A Thesis

entitled

"Synthetic Studies in the Tetracycline Field"

submitted to the

University of Glasgow

in part fulfilment

for the Degree of Doctor of Philosophy

in the Faculty of Science

by C.T. Bedford, M.Sc. (Manchester)

Chemistry Department September 1963

ACKNOWLEDGMENTS

The author wishes to express his thanks to Professor R.A. Raphael, F.R.S., and Dr. A.I. Scott for their friendly and stimulating supervision of the work herein described.

He also wishes to thank:

Mrs. F.M. Lawrie for the measurement of solution infrared spectra;

Mr. J.M. Cameron and his staff for micro-analyses;

Mr. A. Hyslop and Mr. T. Pitt for the construction of various types of oxygenation apparatus;

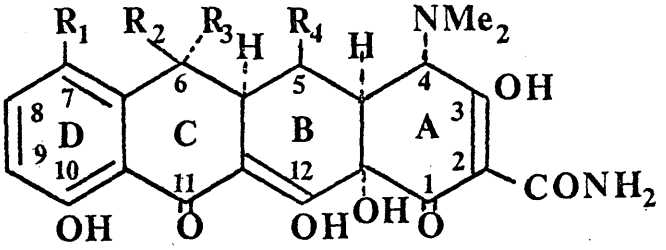
Mr. G. Milmine for his skilful assistance in the preparation of some of the synthetic intermediates.

This work was carried out during the tenure of a grant from Pfizer Limited, Sandwich, Kent.

Contents

Part I

Introduction.



1

Introduction

The tetracyclines are a family of broad spectrum antibiotics produced in the course of the metabolism of various Streptomyces species, or by simple chemical modification of naturally occurring members of the class. They are built upon the pattern of a unique highly oxygenated hydronaphthacene framework (1).

The first of these yellow, crystalline compounds to be discovered was aureomycin,[1] or 7-chlorotetracycline (1, R_1= Cl, R_2= OH, R_3= CH_3, R_4= H), a metabolite of Streptomyces aureofaciens. Two years later, in 1950, the second of the series, terramycin,[2] or 5-hydroxytetracycline (1, R_1= H, R_2R_4= OH, R_3= CH_3), a metabolite of Streptomyces rimosus, was isolated. The preliminary structural studies[3] early indicated two closely related compounds; as well as affording similar degradation products, their ultraviolet spectra were nearly superposable, and their orange-yellow, crystalline hydrochlorides were isomorphous. The publication[4] of the structure of aureomycin and terramycin in 1953 confirmed the predictions as to their similarity, and the name tetracycline was proposed[3] for a structure (1, R_1R_4= H, R_2= OH, R_3= CH_3)

to which they were both simply related. This prototype, tetracycline, was obtained by catalytic hydrogenolysis of 7-chlorotetracycline,[5] and was later discovered as a metabolite of S. aureofaciens;[6] it, too, showed high antibiotic activity.

In a search for further analogues - by modification of the environment in which the antibiotics accumulate and by the use of mutant Streptomycetes - 7-bromo-,[7] 6-demethyl-,[8] and 6-demethyl-7-chlorotetracycline[8] were characterised, the two latter having particular relevance to the mode of biosynthesis of the tetracyclines (see below). 6-Demethyl-6-deoxytetracycline, the simplest biologically active member of the entire group, is so far a non-naturally occurring artefact derived from 6-demethyltetracycline by hydrogenolysis.[9]

The characteristic chemotherapeutic activity of all of these substances is essentially equivalent, and is strictly dependent upon the maintenance of all the constitutional and stereochemical features of the general structure (1). That is to say, while the groups R_{1-4} situated along the upper periphery of the molecule can be varied over a considerable range without effecting a substantial change in antibiotic properties, any modifications elsewhere in the array

leads to a marked decrease or complete loss of biological activity. For example, epimerisation at C_4, a facile reaction at pH 2-6, results in a 95% loss of activity;[10] conversion of the 2-carboxamido substituent to a nitrile grouping in a similar decrease;[11] and removal of the 12a hydroxyl grouping produces complete loss of activity.[11]

Clearly the tetracycline molecule depends for useful activity upon the presence of the sequence of oxygen functions, from the phenolic hydroxyl at C_{10} and the groups at C_{11} and C_{12} through to the ring-A 1,3-diketone grouping.[10] One postulate of the antibiotic activity of the tetracyclines involves the disturbance of certain enzyme systems by preferential chelation of trace metals.[13] The deduction[12] that the 11 and 12 oxygen functions, augmented by the effect of the phenolic hydroxyl at C_{10} are primarily responsible for this chelation effect does not, however, satisfactorily explain the function of the A-ring substituents. One of these substituents, the 2-carboxamido grouping, is strongly intramolecularly hydrogen bonded to the 1,3-diketone system of the A-ring,[14] thereby controlling to a large

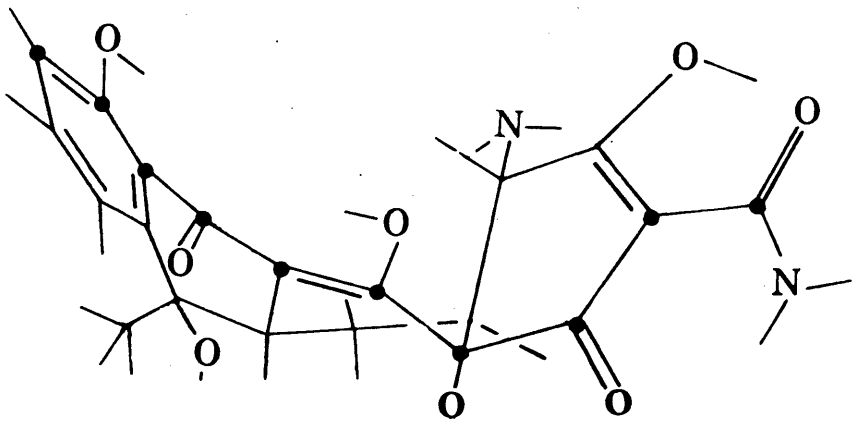


Fig 6

extent the conformation of that ring; it would appear therefore to provide merely an internal spatial requirement. The role of the dimethylamino grouping at C_4 is difficult to rationalise, since complete removal of the grouping results in the loss of only 85% of the activity.[11] The 4-epi-tetracyclines (95% loss of activity) would seem then to possess a dimethylamino grouping which sterically obstructs the antibiotic action of the molecule, and an inspection of a scale drawing[15] (Fig. 6) indicates that the dimethylamino grouping in an epi-compound would indeed be close enough to interact with the oxygen functions at carbons 10, 11 and 12. At the present there seems to be little quantitative correlation between the chelating power of tetracyclines and their antibiotic activity, and this would tend to suggest that such a criterion is not the only controlling factor.

A recent X-ray structural analysis[16] has unequivocally established the relative configuration of the five asymmetric carbon atoms of 7-chlorotetracycline to be as depicted in (1, R_1= Cl, R_2= OH, R_3= CH_3, R_4= H). In the same paper the probable stereochemistry of 5-hydroxytetracycline is suggested

(1, R_1= H, R_2R_4= OH, R_3= CH_3). These results confirm the configuration proposed in 1952 after the preliminary X-ray studies.[17] They also confirm the relationship between the dimethylamino grouping at C_4 and the hydrogen atom at the ring junction C_{4a}, although some of the earlier chemical studies of tetracyclines and their derivatives had seemed to indicate the corresponding trans relationship.[4,18] Later the ease of epimerisation at the 4- position of the tetracyclines was demonstrated, and as a result the correct alternative orientation was proposed on a purely chemical basis.[19,20]

That (1) also expresses the absolute configuration of the tetracyclines, has recently been proposed by Shemyakin et al.[21] In their proof, they utilised rotatory dispersion studies to correlate a synthetic product of known absolute configuration, (-)3-methylphthalide-3-carboxylic acid (2), with a natural degradation product of chlorotetracycline, 7-methoxy-3-methylphthalide-3-carboxylic acid (3) in which the configuration at the 6-position of the natural antibiotic was retained. The natural degradation product was shown to possess the 3R-configuration, opposite to the 3S- configuration of the synthetic product.

A comprehensive review of the chemical and pharmacological properties of the tetracyclines, covering the literature up to 1960, has been published by Shemyakin and his colleagues.[22] Other reviews of the tetracyclines have appeared stressing either their chemistry[23,24] or their general pharmacological properties and use.[25] A recent review,[15] which covers the literature from 1960 to 1962, makes available a valuable and up-to-date addendum to the English translation of the Russian treatise,[26] and includes a lucid summary of the various approaches which have been made to the total synthesis of the tetracyclines.

Confirmatory syntheses of degradation products obtained during the structural elucidation of hydroxytetracycline and chlorotetracycline were announced between 1951 and 1959. In general, the aromatic D-ring survived degradative attack, and confirmatory syntheses were invariably able to make use of a suitably pre-substituted aromatic ring as a corner-stone for the build-up of the diverse degradation products. For example, 3-(4-chloro-7-methoxy-3-methylphthalidyl)-succinic acid,[5](5) derived from 7-chlorotetracycline by methylation and

permanganate oxidation, was synthesised[27] in five stages from 2-cyano-3-methoxyacetophenone,(4).

Attempts towards the synthesis of the tetracyclines themselves, with their five asymmetric centres and their complex array of substituents of different kinds, gained impetus in 1957 with the announcement by several research groups of their projected routes together with their preliminary experimental results on model compounds. The complex stereochemistry and substitution of the tetracycline molecule has inspired many different conceptions of its total synthesis, but, in general, the most successful approach has followed, in broad outline, the probable biogenetic route; various forms of the Claisen condensation are employed in the stepwise fusion of rings C, B, and A to a benzene derivative carrying the ring D substituents and a basis for the construction of ring C. While such routes have indeed led to tetracyclic compounds, the problems of introducing two tertiary hydroxyl groups at C_6 and C_{12a}, and a dimethylamino grouping at C_4, were considerable. Each grouping is very labile - or confers lability on the molecule - and their introduction had necessarily to be delayed (in most routes) until after the vigorous ring-

fusion procedures. In view of these problems it was not surprising that the first major synthetic advances in the field each related to the production of deoxy-dedimethylamino- derivatives. Two research groups, those of Fields, Kende and Boothe in the United States and Muxfeldt in Germany, announced in 1959 the obtention of (±) dedimethylamino-12a-deoxy-5a,6-anhydrochlorotetracyclines (6, R = H;[28] R = CH_3[29]) and (±) dedimethylamino-6,12a-dideoxychlorotetracyclines (7, R = H;[30] R = CH_3[29]), each of which lacked only the three aforementioned features. More recently each group has been able to effect the introduction of the 12a-hydroxyl grouping - Muxfeldt by the action of perbenzoic acid,[31] and the American group by aerobic oxidation in the presence of sodium nitrite.[32] So far the introduction of the other two features has not been reported by these research groups.

Employing a modified approach in which the introduction of the dimethylamino grouping was accomplished prior to construction of the A-ring, Woodward, Conover, Butler, Johnston and Korst were able in August 1962 to announce the first total synthesis of a fully biologically-active tetracycline, (±)6-demethyl-6-deoxytetracycline (8).[33]

The remainder of this section is devoted to a resumé of each of the aforementioned syntheses, commencing with the work of Muxfeldt, proceeding to that of Fields, Kende and Boothe, and finally to that of Woodward and Conover and their collaborators. The lucid, schematic outlines of each of the syntheses (Figs. 1-5) are taken from the comprehensive review article mentioned above,[15] and the ensuing commentary is made with reference to these figures.

The synthesis of (±) dedimethylamino-6,12a-dideoxy-decarboxamido-7-chlorotetracycline (10) by Muxfeldt and his group is outlined in Fig. 1. The selective crystallisation, from a mixture of isomers, of the tetralone (9) possessing the stereochemical requirements of two of the three asymmetric carbon atoms of the synthetic objective (10), was a key step in the synthesis. In the synthesis of (±)dedimethylamino-12a-deoxy-5a,6-anhydro-7-chlorotetracycline (12) the unresolved mixture of the tetralone (9) and its isomer was aromatised to an optically inactive naphthol derivative (11), which was then elaborated to (12), as outlined in Fig. 2.

The corresponding two syntheses by the United States group of Fields, Kende and Boothe followed a

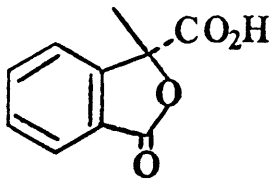
similar pattern to that of the German group, in that each synthesis derived from a common intermediate, a substituted tetralone (13). Aromatisation of this tetralone, with subsequent elaboration, led to the synthesis of (±) dedimethylamino-6-demethyl-12a-deoxy-5a,6-anhydro-7-chlorotetracycline (14), as outlined in Fig. 3. In the synthesis of (±) dedimethylamino-6-demethyl-6,12a-dideoxy-7-chlorotetracycline (16), outlined in Fig. 4, the tetralone (13) was ring closed to a mixture of isomeric tricyclic acids, from which the important tricyclic acid (15) possessing the desired stereochemistry was selectively crystallised.

The Woodward-Conover synthesis is outlined in Fig. 5. The recurring feature of the four syntheses described above, a substituted tetralone, again appeared early in the sequence (17). The important introduction of the dimethylamino substituent was the highlight of the synthesis. As anticipated, the tricyclic intermediate (18) was found to possess the high susceptibility to the addition of nucleophiles which is a usual characteristic of substances containing an olefinic bond flanked by electron-withdrawing substituents. Thus when it was dissolved

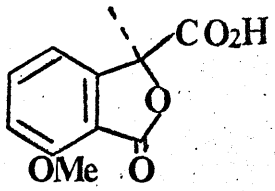
in liquid dimethylamine, addition took place with great facility to give the desired base (19). However, the extraordinarily readily reversible nature of this reaction prevented the simple isolation of (19); although, under properly chosen conditions, a crystalline sample was rapidly isolated for spectral characterisation purposes. The instability of the addition product is due to the conjunction of a carbonyl group and a β-situated dimethylamino group, and by carrying out a borohydride reduction of (19) as soon as it was formed, the corresponding stable, crystalline alcohol (20) was isolated in 60% overall yield. The stereoselectivity of the changes was marked; of the three asymmetric centres formed during the reaction, two were shown to possess the stereochemistry of the synthetic objective (23). The third centre, in view of its observed ready epimerisation, was known to be amenable to orientation in the desired sense at a later stage of the synthesis, and was unassigned.

A further feature of the synthesis was the elegant use made of the sodium hydride/dimethylformamide combination as a means of effecting several of the more difficult anionoid condensation reactions. The final

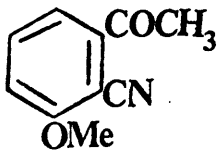
cyclisation reaction, (21)→ (22), was a case in point. Treatment of (21) with sodium hydride/ dimethylformamide first at room temperature, and then after addition of methanol, for a short time at 120°C gave (22) in 20% yield. The formation of (22) was, at first, considered improbable, and indeed only by rigorously adhering to the above conditions was cyclisation effected.


CO2H
O
O

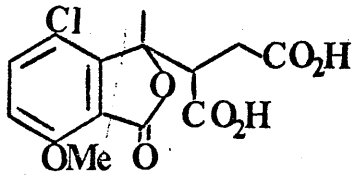
2


CO2H
O
OMe
O

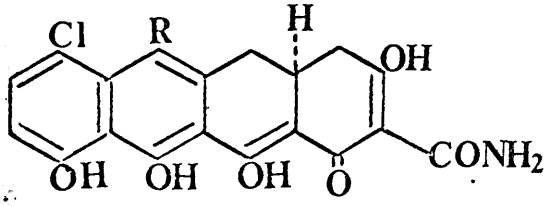
3


COCH3
CN
OMe

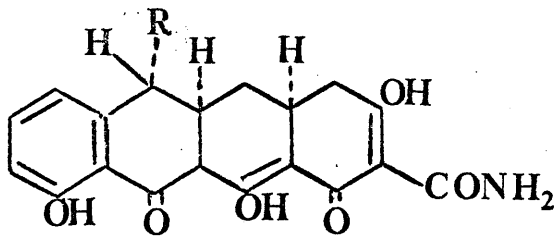
4


Cl
CO2H
O
CO2H
OMe
O

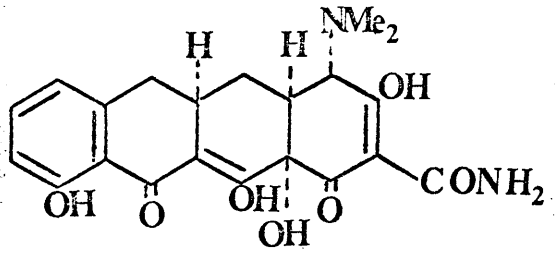
5


Cl
R
H
OH
CONH2
OH
OH
OH
O

6


H
R
H
H
OH
CONH2
OH
O
OH
O

7


H
H
NMe2
OH
CONH2
OH
O
OH
OH
O

8

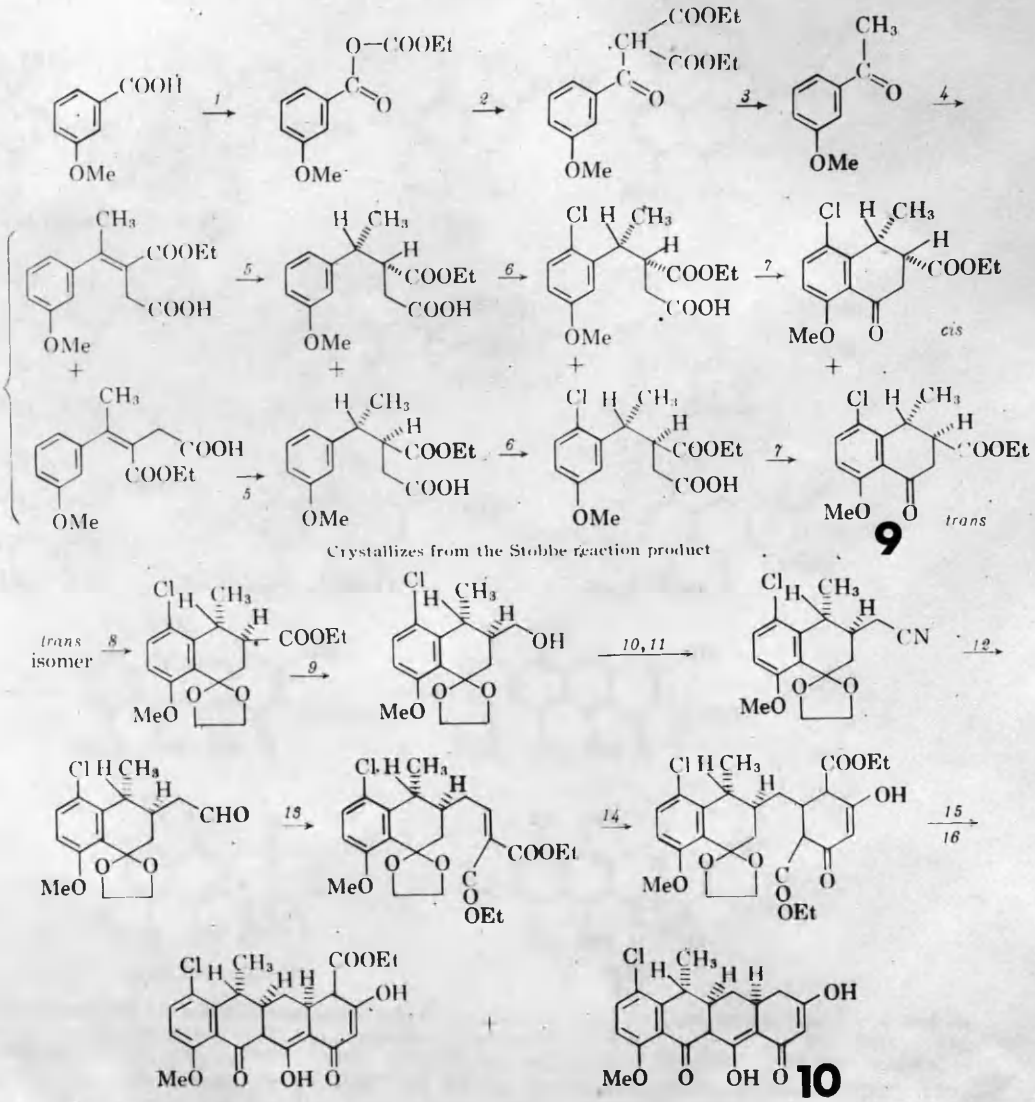


Fig. 1.—Synthesis of (±)-dedimethylamino-6,12a-dideoxy-decarboxamido-7-chlorotetracycline

Reagents: (*1*), ClCOOEt/N-methylmorpholine/benzene; (*2*), (EtOOC—CH—COOEt) Mg^{++} (OEt); (*3*), aq. H_2SO_4/AcOH/heat; (*4*), diethyl succinate/NaH; (*5*), H_2/Ni; (*6*), 1 equiv. Cl_2/CCl_4; (*7*), polyphosphoric acid; (*8*), ethylene glycol; (*9*), $LiAlH_4$; (*10*), methanesulfonyl chloride/pyridine; (*11*), KCN/dimethylformamide/water; (*12*), $LiAl(OEt)_3H$; (*13*), diethyl malonate/AcOH/piperidine; (*14*), Na^+ (CH_3CO. CH COOEt)/ether/reflux; (*15*), aq. HCl; (*16*), NaH/anisole.

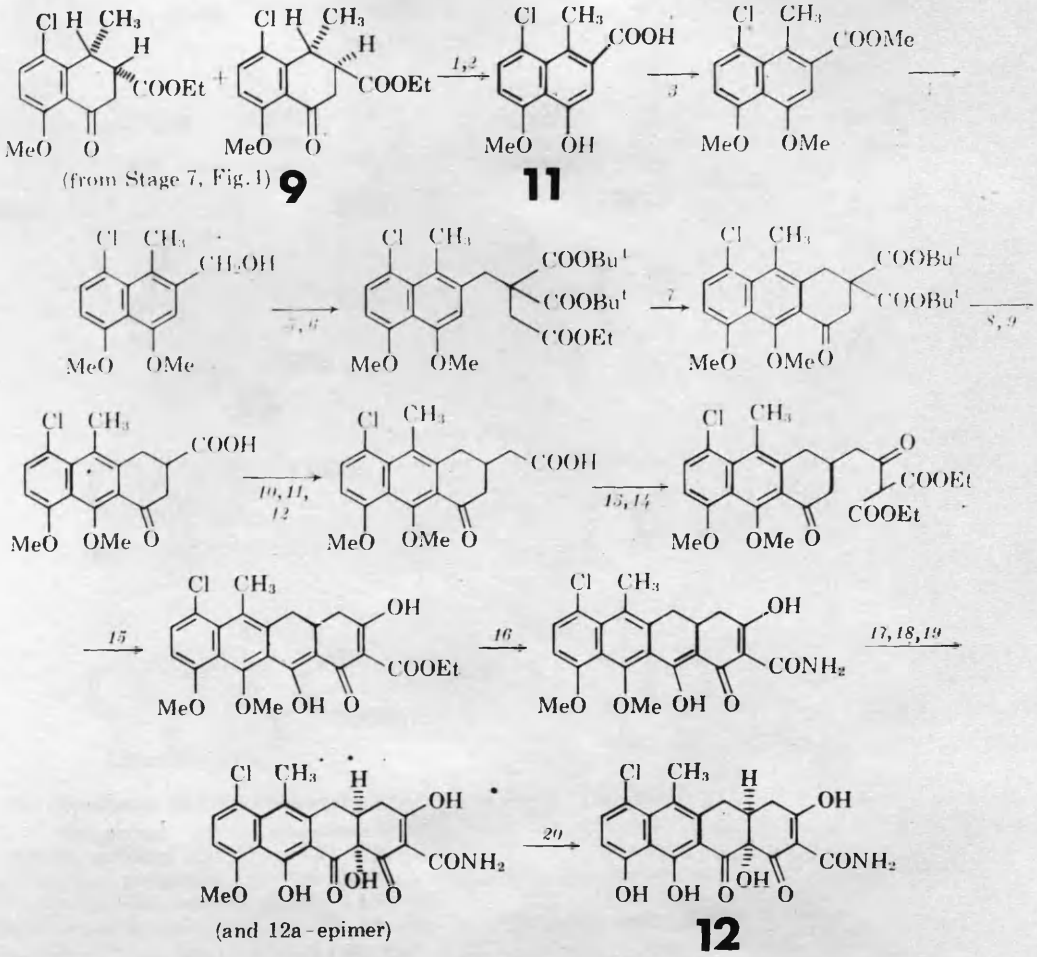


Fig. 2.—Synthesis of (±)-dedimethylamino-12a-deoxy-5a,6-anhydro-7-chlorotetracycline ( ) and its conversion into (±)-dedimethylamino-5a,6-anhydro-7-chlorotetracycline . Reagents: (*1*), Br_2/ether/500-watt lamp; (*2*), NaOH/MeOH; (*3*), CH_2N_2; (*4*), $LiAlH_4$; (*5*), PBr_3; (6), Na^+(EtOOC—CH_2—$\bar{C}$(COOBu^t)$_2$); (*7*), polyphosphoric acid; (*8*), dil. aq. NaOH; (*9*), diethyl phthalate/170°; (*10*), PCl_5 or oxalyl chloride; (*11*), CH_2N_2; (*12*), benzyl alcohol/180°; (*13*), PCl_5; (*14*) Mg^{++}(EtOOC—$\bar{C}$H—COOEt)$_2$; (*15*), NaH/anisole; (*16*) NH_3/NaOMe—MeOH; (*17*), HCl/AcOH; (*18*), CH_2N_2; (*19*), $PhCO_3H$/$CHCl_3$; (*20*), HCl/AcOH.

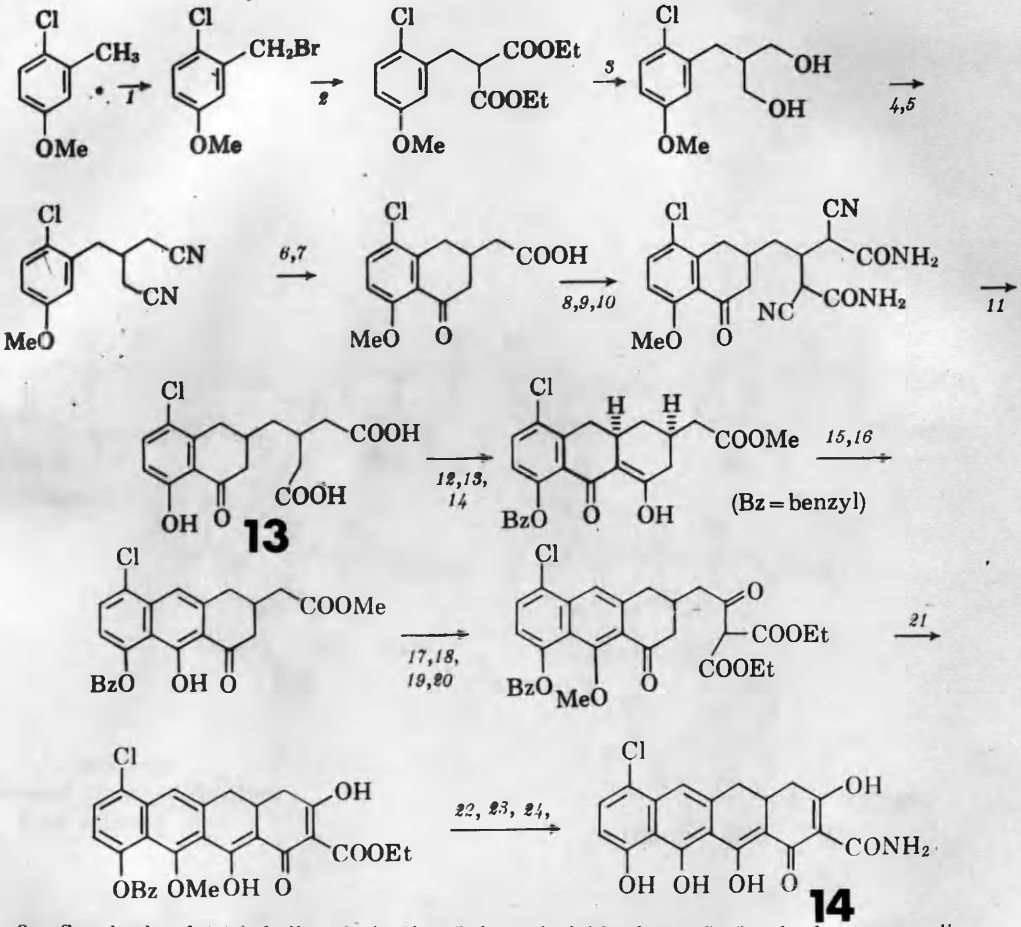


Fig. 3.—Synthesis of (±)-dedimethylamino-6-demethyl-12a-deoxy-5a,6-anhydro-tetracycline

Reagents: (*1*), N-bromosuccinimide/peroxide; (*2*), $Na^+(EtOOC—\bar{C}H—COOEt)$; (*3*), $LiAlH_4$; (*4*), methane sulfonyl chloride; (*5*), CN^-; (*6*), OH^-; (*7*), polyphosphoric acid; (*8*), oxalyl chloride; (*9*), Rosenmund reduction (5% Pd–$BaSO_4$); (*10*), cyanoacetamide/piperidine; (*11*), concd. HCl/AcOH; (*12*), benzyl chloride/boiling alkali; (*13*), $MeOH/H_2SO_4$; (*14*), NaH/toluene; (*15*), Br_2/NaOAc; (*16*), collidine (dehydrobromination); (*17*), Me_2SO_4/K_2CO_3; (*18*), mild alkaline hydrolysis; (*19*), ethyl chloroformate/NEt_3; (*20*), $Mg^{++}(\bar{O}Et)(EtOOC—\bar{C}H—COOEt)$; (*21*), NaH/toluene; (*22*), H_2/10% Pd–C; (*23*), $HCOO^-NH_4^+$/140°; (*24*), boiling concd. HCl/AcOH.

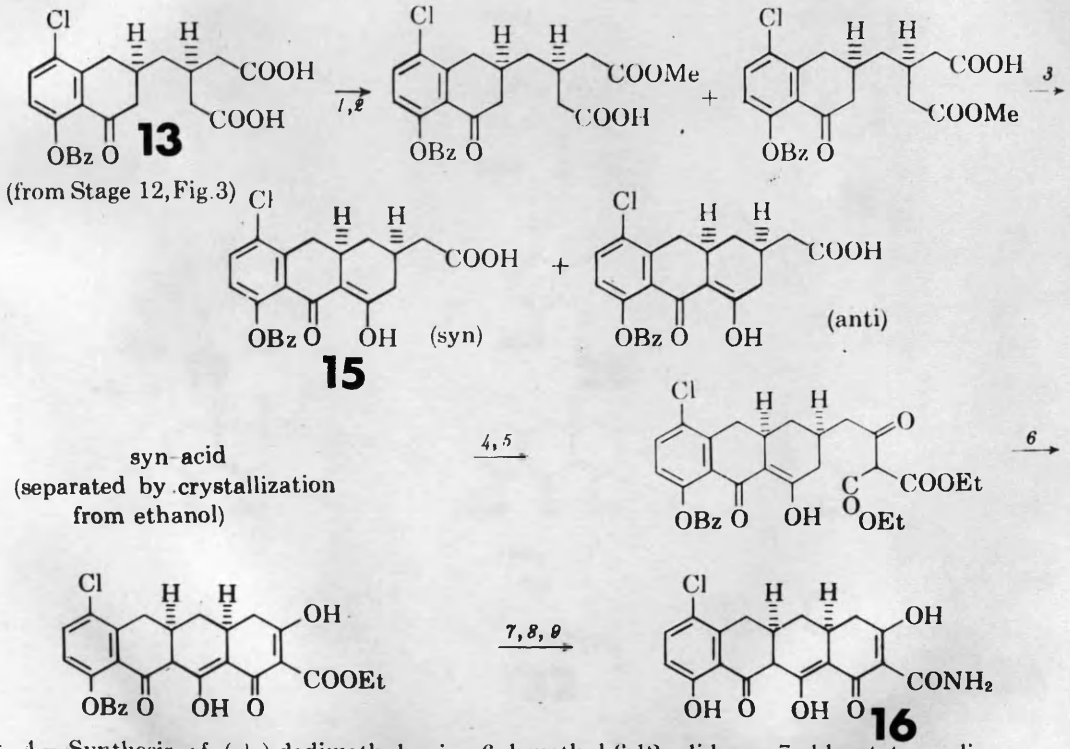


Fig. 4.—Synthesis of (±)-dedimethylamino-6-demethyl-6,12a-dideoxy-7-chlorotetracycline
Reagents: (*1*), Ac_2O/reflux/1 hr.; (*2*), NaOMe/MeOH; (*3*), NaH/toluene; (*4*), ClCOOEt/NEt_3; (*5*) Mg^{++} (EtOOC—CH—$COOEt_2$); (*6*), NaH/toluene; (*7*), H_2/10% Pd-C; (*8*), $HCOO^-NH_4^+$/140°; (*9*), Aq.HCl.

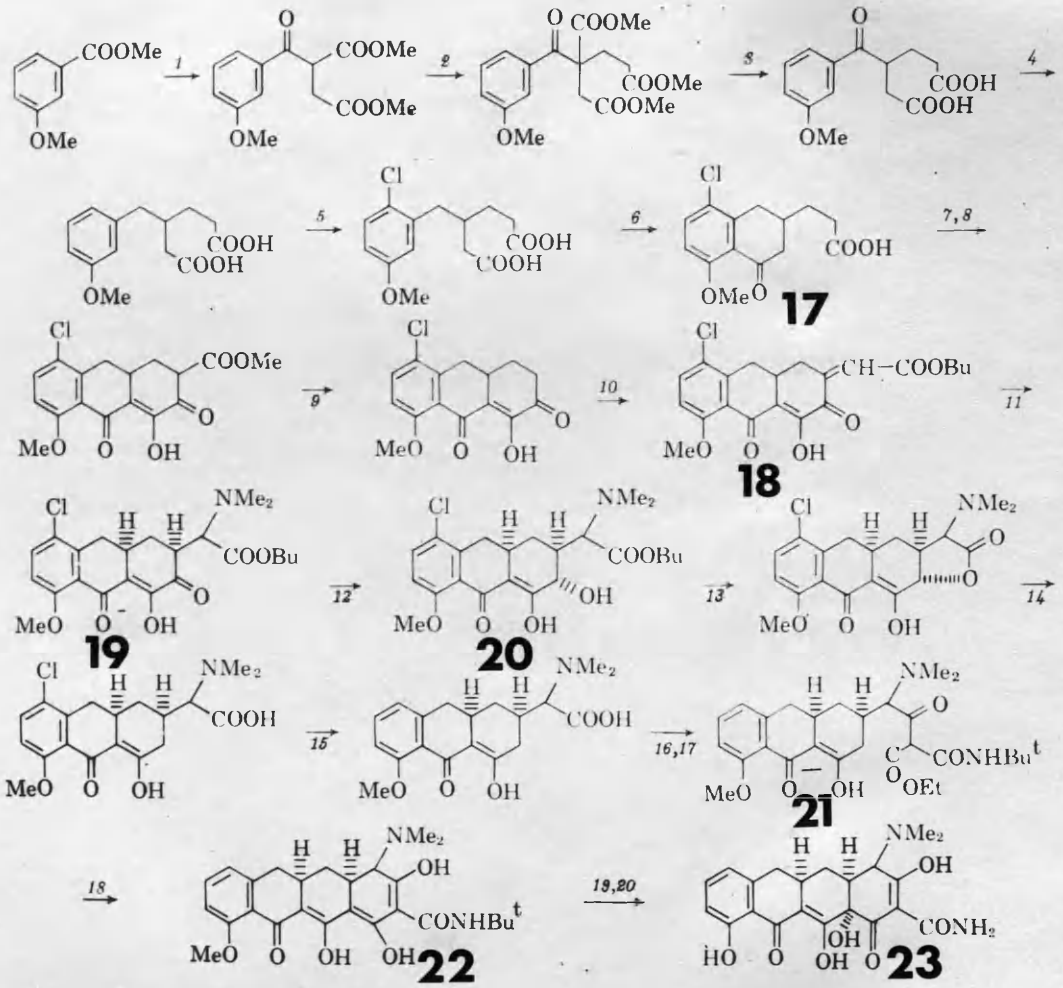


Fig. 5.—Synthesis of (±)-6-demethyl-6-deoxytetracycline. Reagents: (*1*), dimethyl succinate/NaH/dimethylformamide; (*2*), methyl acrylate/Triton B; (*3*), hot aq. H_2SO_4/AcOH; (*4*), H_2/Pd–C/AcOH/200 p.s.i.; (*5*), Cl_2/AcOH/15°; (*6*), HF/15°; (*7*), esterify; (*8*), dimethyl oxalate/1 equiv. MeOH/NaH/dimethylformamide; (*9*), hot aq. HCl/AcOH; (*10*), $Mg^{++}(\bar{O}Me)_2$/*n*-butyl glyoxalate/toluene; (*11*), Me_2NH/−10°; (*12*), $NaBH_4$/diglyme/low temp.; (*13*), toluene sulfonic acid/toluene; (*14*), Zn/HCOOH; (*15*), H_2/Pd–C/EtOH/NEt_3; (*16*), $ClCOOPr^i$; (*17*), $Mg^{++}(EtOOC-\bar{C}H-CONHBu^t)_2$; (*18*), NaH/dimethylformamide/120°; (*19*), hot 48%, aq. HBr; (*20*), $CeCl_3/O_2$/dimethylformamide/MeOH/pH 5.

Part II

The Biosynthesis of the Tetracyclines.

The Biosynthesis of the Tetracyclines

The biosynthesis of the tetracyclines has been investigated in some detail in the hope of elucidating the complete pathway with the aim of using the knowledge thus gained to produce "tailor-made" tetracyclines with possibly enhanced activity.

The production of 7-chlorotetracycline by S. aureofaciens is dependent upon the presence of ionic chlorine in the nutrient medium, but the total yield of tetracyclines produced under conditions of low halide concentration is unchanged since tetracycline itself then forms in increased amount.[34] Thiocyanate, added to the usual S. aureofaciens nutrient medium, also inhibits 7-chlorotetracycline synthesis but the total yield of tetracyclines is again unchanged.[34,35] The same organism can be induced to synthesise 6-demethyl-7-chlorotetracycline in addition to 7-chlorotetracycline by introduction of sulphonamides into the nutrient medium;[36] the proportion of the 6-demethyl compound formed under these conditions can be made smaller by the addition of methionine to the medium. Halogenation and methylation are thus shown to be late steps in the biosynthetic sequence.

The examination of the fermentation liquors of various mutant strains of tetracycline-producing organisms has separately yielded three new classes of compound, each closely related to the tetracyclines. The first of the class reported were the two 6-demethyltetracyclines (20, R = H; R = Cl),[37] which were later obtained by the introduction of sulphonamides into the nutrient medium of normal strains (see above). Only one representative of the second class, 7-chloro-5a,11a-dehydrotetracycline (21), has been reported.[38] Its conversion to 7-chlorotetracycline by the normal strain of S. aureofaciens, thus showing it to be a direct precursor, has been demonstrated.[39] Three representatives of the third class, the 2-acetyl-2-decarboxamidotetracyclines, have been described: 7-chloro-(22, R_1= Cl, R_2= H),[40] 5-hydroxy-(22, R_1= H, R_2= OH)[41] and the tetracycline analogue itself (22, R_1R_2= H).[40]

The tetracyclines display features indicative of an "acetate" origin.[42,43] The oxygenation pattern is generally consistent as are the points of occurrence of methyl groups and halogen atoms. That they are derived almost entirely from "acetate" has been

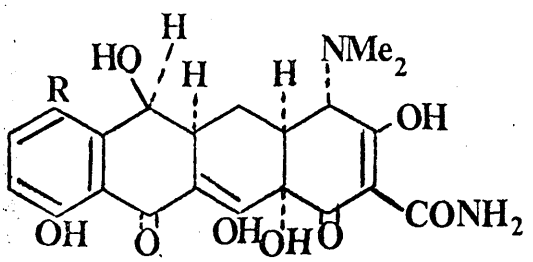
demonstrated by Birch, who obtained radioactive 5-hydroxytetracycline after feeding $^{14}CH_3CO_2H$ to the fermentation liquors.[44] The labelling-patterns of the degradation fragments of the radioactive 5-hydroxytetracycline were entirely consistent with formation of the ring skeleton (at least from C_5 to C_{12}) by head to tail linkage of "acetate" units. The origin of the carbon atoms of part of the A-ring (carbon atoms 2, 3, 4 and 4a) and the carboxamido side-chain was less clear, mainly due to the difficulty of obtaining degradation fragments uniquely attributable to these carbon atoms. 2-^{14}C-Labelled glutamic acid has yielded labelled 5-hydroxytetracycline, and has been tentatively considered as a possible precursor of these carbon atoms and the two nitrogen atoms.[45] On the other hand, the labelling-patterns obtained from the $^{14}CH_3CO_2H$ experiments are not inconsistent with at least a partial "acetate" derivation of ring-A.[44]

The addition of [Me-^{14}C]-methionine to the fermentation liquors also yielded radioactive 5-hydroxytetracycline, degradation experiments demonstrating that the radioactivity had been wholly incorporated into the 6-methyl group and the methyl groups of the dimethylamino grouping.[44]

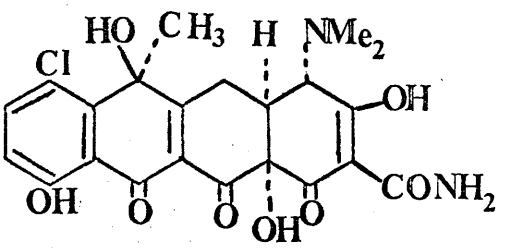
The above findings have been summarised by Birch in a schematic representation of the probable biosynthesis (Fig. 7).[44] The introduction of the C_1 units and of the oxygen functions at 5,6 and 12a are modifications of the main scheme, as indicated in Fig. 7. The 5a,11a-dehydrotetracycline, shown to be a direct precursor of a tetracycline (see above), fits in well with the "acetate" derivation, and Birch has suggested that the double bond in this compound is a remnant of an aldol ring closure which produces the B/C ring junction.[44]

Recent studies on the biogenesis of fatty acids[46] as well as other metabolites[47] have indicated the true chain-building intermediate to be "malonate" rather than "acetate".[48] In view of these findings, the skeleton of the tetracyclines is now considered to be derived from nine "malonate" units with the half-amide of "malonate" as the chain initiator[41] (cf. Fig. 8). In the case of the closely-related 2-acetyl-2-decarboxamidotetracyclines, an "acetate" unit becomes the chain initiator.[41] In preliminary support of these proposals, Gatenbeck has recently reported the isolation of labelled 5-hydroxytetracycline after feeding carboxyl-labelled malonic acid.[49]

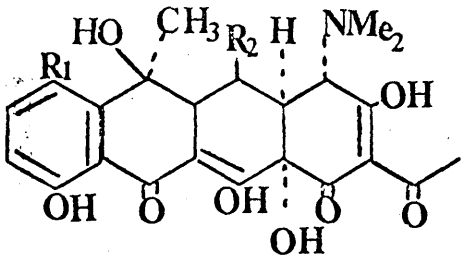
In the same series of experiments, ^{14}C-labelled bicarbonate was incorporated into 5-hydroxytetracycline, subsequent degradation demonstrating an almost specific (>90%) incorporation into the 2-carboxamido substituent.[49]



20



21



22

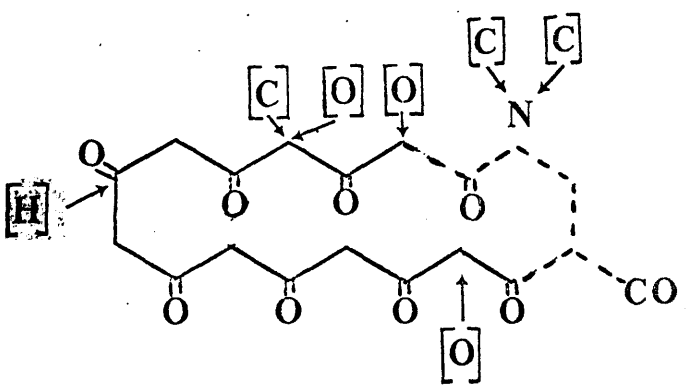


Fig. 7

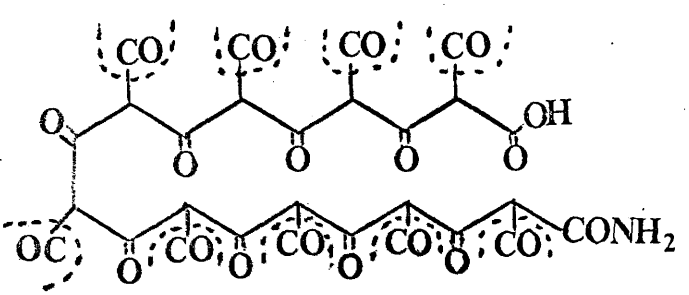


Fig. 8

Part III

Hydroperoxides Derived from Phenols as Possible Biosynthetic Intermediates. The Preparation of 7-chloro-6-deoxy-6-hydroperoxy-5,5a-dehydrotetracycline and its Significance in the Biosynthesis of the Tetracyclines.

Hydroperoxides Derived from Phenols as Possible Biosynthetic Intermediates. The Preparation of 7-chloro-6-deoxy-6-hydroperoxy-5,5a-dehydrotetracycline and its Significance in the Biosynthesis of the Tetracyclines

An important late step in the biosynthesis of the tetracyclines, whose skeletons are largely evolved from head-to-tail linkage of "acetate" units[44] (see above), is the introduction of hydroxyl functions at positions 5,6 and 12a, (cf. Fig. 7). Laboratory analogies for 12a-hydroxylation of the corresponding deoxytetracyclines have been accomplished; in which perbenzoic acid,[31] acrobic oxygen in the presence of sodium nitrite,[32] and oxygen in the presence of platinum[50] have each been effective reagents. The mechanism of biochemical hydroxylation at position 6 merits particular attention in view of the isolation of 7-chloro-5a,11a-dehydrotetracycline (21) from a mutant of Streptomyces aureofaciens[38] and the demonstration that this metabolite is a precursor of 7-chlorotetracycline.[39]

The structural features of the rings B and C of 7-chloro-5a,11a-dehydrotetracycline constitute a p-quinol system which is novel in a naturally-

occurring organic compound, though p-quinols and their closely-related analogues the o-quinols, were first prepared in the laboratory at the turn of the century.[53] The quinol grouping is usually extremely labile (this presumably accounts for its rarity in naturally-occurring systems) but in the 7-chloro-5a,11a-dehydrotetracycline molecule (21) the p-quinol system is uniquely stabilised by an especially-favourable structural environment. The explanation of this unique stabilisation is best held over, however, until a resume of the preparation and properties of the o- and p-quinols has been presented.

p-Quinols were first obtained by the acid-catalysed rearrangement of p-alkyl-phenylhydroxyl-amines,[51,52] e.g. (25). Bamberger showed that the same compounds were available by the oxidation of p-alkyl phenols with Caro's acid (permonosulphuric acid) in the presence of excess magnesium carbonate.[53] In this way, he converted e.g. p-cresol (23), to p-toluquinol (24).[53] He also found the method adaptable to the preparation of o-quinols *via* the oxidation of o-alkyl phenols.[53] The preparation and chemistry of the o- and p-quinols have been reviewed in a recent progress report by London,[54] who has been able to collate the work of Bamberger with the more recent methods of preparation. The most important

newly-discovered route to the o- and p-quinols proceeds by the C-acetoxylation of phenols with lead tetra-acetate in acetic acid, a reaction discovered in 1950 by Wessely.[55] The reaction has since been employed extensively,[54,55,56,65] and is conveniently known as the "Wessely Oxidation." The products of "Wessely Oxidation" are o- and p-quinol acetates; thus o-cresol (27) and p-cresol (23) are converted to o-toluquinol acetate (28) and p-toluquinol acetate (26) respectively.[56] The free o- and p-quinols are obtainable from the quinol acetates by mild alkaline hydrolysis or base-catalysed methanolysis;[57,58] the Wessely C-acetoxylation reaction thus provides an invaluable alternative to the less effective Bamberger method, which has given poor or negative results with a number of phenols.[59] (A recent amelioration however involves dispensing with the awkward Caro's acid; thus direct hydroxylation of 1-methyl-2-naphthol(29) to the o-quinol (30) by the action of peracetic acid has been reported by Woodward and Doering.[60] This technique, however, seems to have been overlooked in most of the recent papers on the subject - understandably perhaps, since it appeared only in a footnote of a paper describing the synthesis of quinine.)

Recently the use of lead tetraacetate in solvent methanol in the presence of boron trifluoride has provided a method of preparation of the quinol methyl ethers.[61] In this way, p-cresol yielded p-toluquinol methyl ether (31), and oestrone the interesting p-quinol methyl ether, (32).[61] The latter reaction provides a very feasible in vitro analogy for the observation that 6-hydroxytetralin (33), when used as a model for oestradiol, was metabolised by rat liver microsomes to its corresponding p-quinol (34.)[62] Another example of a quinol ether derived from a natural product has been reported - that of the "dimeric" o-quinol ether (36) which results from potassium ferricyanide oxidation of α-tocopherol (35).[63]

p-Quinols are subject to rearrangement[54] in either aqueous acid [Chart 1] or aqueous alkali [Chart 2], as is illustrated by the reactions of p-toluquinol. A hydroquinone derivative is thereby formed by migration of the alkyl group. On the other hand, an acetoxyl group migrates and a derivative of resorcinol is formed when the p-quinol is exposed to the conditions of Thiele acetylation (acetic anhydride in presence of concentrated sulphuric acid), and a similar change - to a resorcinol monoacetate - is induced in the p-quinol acetate by ethereal boron

trifluoride [54] [Chart 3]. The o-quinols and o-quinol acetates rearrange in an analogous manner to resorcinol derivatives.[65] A similar p-quinol-rearrangement has been invoked by Witkop to explain the biological oxidation of tyrosine to homogentisic acid (38),[64] and he was able to simulate the final stages of his proposed sequence by the base-catalysed rearrangement of the p-quinol, (37; H for Ac). The rearrangement was conveniently demonstrated by treatment of the corresponding p-quinol acetate (37) (prepared via "Wessely Oxidation") with mild aqueous alkali, which consecutively effected hydrolysis and rearrangement. The true intermediate in the sequence tyrosine → homogentisic acid is seen as the p-quinol, (39), or its closely-related hydroperoxide, (40), whose plausible mode of rearrangement is shown in Chart 4.[64] In an alternative hypothesis,[66] an intermediate spirolactone (41) is considered to rearrange intermolecularly to homogentisic acid.

Here we may briefly reconsider the subject of the stabilisation of the p-quinol system in the 7-chloro-5a,11a-dehydrotetracycline molecule, (21). As has been shown above, p-quinols are susceptible,

in general, to molecular rearrangement by both mild acid and mild base. However, it is evident (cf. Charts 1 and 2) that the rearrangement cannot occur if the p-quinol is substituted at both of the vicinal carbon atoms, since there would be no proton to lose in the final aromatisation step. It is this feature of the p-quinol system in (21) (the substitution at both vicinal carbon atoms) which provides the "especially-favourable structural environment" leading to the unique stabilisation briefly mentioned above.

That the products of the "Wessely Oxidation" (lead tetraacetate/acetic acid) of phenols are best explained in terms of a free radical mechanism, has been shown by Wessely[56,65] and Cavill.[67] The initial step is envisaged to involve the dehydrogenation of the phenol to give a mesomeric radical, which may then react with an acetoxyl radical ($CH_3CO.O^{\bullet} \equiv AcO^{\bullet}$) to give the C-acetoxylated products. The reaction-scheme is illustrated by the oxidation of p-cresol [Chart 5]. Here the p-acetoxylated product is formed in preference to the o-acetoxylated product in view of the relative stability (tertiary > secondary) of

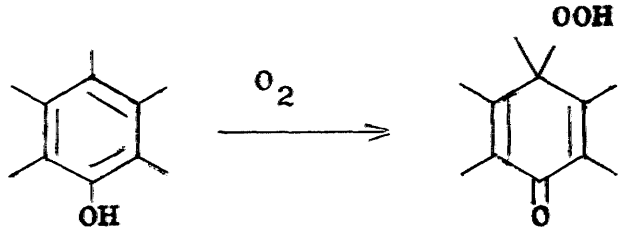
the respective intermediate radicals. Although the large excess of acetoxyl radicals deliberately employed in the "Wessely Oxidation" of phenols leads almost solely to "monomeric" acetoxylated products, the possibility of obtaining, in addition, non-acetoxylated "dimeric" products has been clearly demonstrated by employing lead tetraacetate with benzene as solvent;[67] under these conditions one of the products isolated, (42), results from the dimeric coupling of the mesomeric phenol radical formed initially [Chart 6]. This intermolecular free-radical coupling of phenols is also induced by many other oxidising agents, e.g. potassium ferricyanide, Fenton's reagent, ferric chloride, lead dioxide, and manganese dioxide (cf.[69]), and there is considerable evidence (e.g.[68]) that coupling reactions of this type play an important role in the biosynthesis of complex phenols, a topic whose far-reaching consequences have been discussed by Barton and Cohen.[69]

The "monomeric" products of the free radical oxidation of phenols; however, appeared to us to play just as important a role in the biosynthesis of many other naturally-occurring compounds, especially those which are known to derive from a carbon

skeleton *via* the unspecified "biological" introduction of oxygen. The unique tetracycline metabolite, 7-chloro-5a,11a-dehydrotetracycline (21) provides a signal and illustrative example of the type, and the biological formation of homogentisic acid (38) from tyrosine appears also to involve the similar intervention of a *p*-quinol derivative, as was demonstrated by Witkop.[64]

In Witkop's paper,[64] passing mention was made of a hydroperoxy analogue of a *p*-quinol (quinol-hydroperoxide; 40), which was considered a closer analogue of the true intermediate in the biological sequence tyrosine → homogentisic acid. We have followed up, extended, and generalised this possible *in vivo* intervention of a hydroperoxy analogue of a quinol. We would like to put forward the proposition that **in the cases of a large number of naturally-occurring organic compounds where oxygen is introduced "biologically", an intermediate phenol undergoes direct interaction with molecular oxygen** -

most probably involving a free radical mechanism - to give a quinol-hydroperoxide. e.g.



The hydroperoxide may - and normally would - subsequently undergo an intramolecular rearrangement, the nature of the product or products being uniquely predictable on the basis of analogies with the known rearrangement products of alkyl hydroperoxides (cf.[70,71]). In favourable cases, i.e. where the structural environment of the hydroperoxides precludes possible rearrangement, the hydroperoxide would be isolable. It is thus envisaged that the standard procedures of isolation of appropriate naturally-occurring compounds - or the utilisation of more sophisticated techniques - will lead to the characterisation of naturally-occurring quinol-hydroperoxides.

There is considerable evidence for the feasibility of such a hypothesis, and indeed several in vitro preparations of quinol-hydroperoxides (significantly

under conditions which approximate to an *in vivo* environment) have been reported (cf.[70]). For example, 9-alkyl-10-anthranols (43) under neutral conditions are autoxidised to 9-alkyl-9-hydroperoxy-10-anthrones (44),[72] and 2,4,6-tri-*t*-butylphenol (45) under alkaline conditions is antoxidised to a mixture of the *o*-quinol-hydroperoxide (46) and the *p*-quinol-hydroperoxide (47).[73] Evidence for the *in vivo* rearrangement of the quinol-hydroperoxides has been adumbrated by Witkop[64] in his elegant simulation of the biosynthesis of homogentisic acid (*vide supra*). Moreover, in the light of the generalised hypothesis outlined above, the biosynthesis of many natural products, whose "biological introduction of oxygen" has hitherto not been rationalised, may be clarified (e.g. tropolones; see below). The evidence for the *in vitro* rearrangement of quinol-hydroperoxides is, in the main, fragmentary. Coppinger[74] appears to have carried out an acid-catalysed rearrangement of a *p*-quinol-hydroperoxide (48), but this report only mentions that the nature of the product differed from that of the rearrangement under the same conditions of the corresponding *p*-quinol (49), which yielded the benzyl acetate, (50)[75] (The

mechanism[75] of this interesting reaction is considered later in a section devoted to the tetracyclines). Kharasch and Joshi[76] have treated the same p-quinol-hydroperoxide (48) with ethanolic potassium hydroxide, and obtained a 20% yield of an uncharacterised "dimeric" compound and 40% of the corresponding p-quinol (49). The alkali-treatment of t-alkyl hydroperoxides likewise[77] gives the corresponding hydroxy compounds, with concomitant evolution of oxygen.[77] By this analogy, evolution of oxygen most probably occurred in the experiment of Kharasch and Joshi, although it was not reported. This obtention of a similar product of rearrangement of a quinol-hydroperoxide and of an alkyl hydroperoxide provided significant - albeit solitary - support for many of our own proposals. Thus, in a number of cases (see below) we have anticipated that the extraordinary variety of plausible rearrangement reactions undergone by alkyl (particularly aralkyl[71]) hydroperoxides,[70] occurs in an analogous manner in the case of quinol-hydroperoxides. Although our proposals more significantly relate to the rearrangement reactions of quinol-hydroperoxides in vivo, presumably brought about by the agency of specific enzymes (cf.[78]), it

is anticipated that most, if not all, of these rearrangements will yield to *in vitro* simulation by the use of suitable chemical reagents in the laboratory.

The ensuing illustration and elaboration of these proposals is most conveniently considered sequentially according to multiplicity of ring-system - i.e. phenols (monocyclic), naphthols (bicyclic) and anthranols (tricyclic), will precede consideration of the tetracyclines (tetracyclic). The experimental observations which we have carried out in an attempt to corroborate these proposals are juxtaposed appropriately, and provide, in some cases, convincing *in vitro* evidence for the feasibility of the operation of similar processes *in vivo*.

Monocyclic Systems (Phenols).

The products of the free radical oxidation of phenols may be predicted with a fair degree of accuracy by a consideration of the relative stabilities (tertiary > secondary > primary) of the initially-formed mesomeric phenol radicals, (cf. Wessely's work on the lead tetraacetate oxidation of variously substituted phenols lucidly tabulated by London[54]). Accordingly the products of the interaction of oxygen with phenols will depend to a large extent on the substitution pattern of the phenol:

para-Oxygenation.

A general expression for para-oxygenation is portrayed in Chart 7. In vitro syntheses of quinol-hydroperoxides of type (51) have been reported, e.g. (47) and (48), and these have been mentioned previously.

Witkop's postulated pathway for the biological conversion of tyrosine to homogentisic acid (38) involves the possible intervention of a quinol-hydroperoxide of type (51) (R = $CH_2.CO.CO_2H$), and this has been considered previously.

ortho-Oxygenation.

A general expression for ortho-oxygenation is portrayed in Chart 8. The in vitro synthesis of a quinol-hydroperoxide of type (52) has been reported, e.g. (46), and this has been mentioned previously.

The concept of ortho-oxygenation of an ortho-alkyl phenol offers a novel and particularly attractive rationale of the biosynthesis of tropolones. The proposed scheme involves the reduction of the quinol-hydroperoxide (52, R = CH_2X) to the corresponding quinol (53), followed by rearrangement to

the tropolone. The X grouping represents a "good leaving group" such as phosphate or pyrophosphate.

The scheme is exemplified by the portrayal of the elements of the biosynthesis of colchicine, (55, R = Me).[79] It is of note that (54) represents an alternative form of the non-tryptophan derived segment of the Rauwolfia alkaloids, cf.[80].

Bicyclic Systems (Naphthols).

This section is devoted to the simpler naphthol derivatives. A series of more complex naphthol derivatives, the anhydrotetracyclines, are considered in a later section (Tetracyclic Systems).

The simpler naphthol derivatives constitute readily-available substrates which are very convenient to use as models for the more complicated anhydrotetracyclines. After our preliminary success in preparing a tertiary p-quinol-hydroperoxide of a tetracycline derivative (see later), we focussed our attention on the preparation of the secondary p-quinol-hydroperoxides of simple analogues - hoping in this

way to provide an *in vitro* analogy for the naturally-occurring 6-demethyltetracyclines (samples of which were unavailable during the earlier part of our work).

The simplest analogue to hand was α-naphthol itself. We knew it to be susceptible to free radical oxidation, since Wessely had obtained the diacetoxy-enone derivative, (57), *via* lead tetraacetate oxidation.[81] Accordingly we subjected α-naphthol (56) in benzene to our photo-oxygenation procedure (for details, see later) in the hope that we might obtain one or both of the quinol-hydroperoxides, (58), (58A). We were aware of only one precedent for such a reaction; that was the product obtained *via* lead tetraacetate oxidation of methoxy-naphthol, to which had been assigned[82] the analogous *secondary* p-quinol acetate structure (59). The oxygenation of α-naphthol afforded us little encouragement, for we were unable

to detect the presence of a hydroperoxide (colour-test; see later) even after prolonged treatment (21 days). However, there was certainly evidence of reaction, for a sample of the reaction mixture taken after 5 days showed a new maximum (*inter alia*) at $\nu = 1710 cm^{-1}$. An ultraviolet spectrum of this sample exhibited the spectrum of α-naphthol, indicating the presence of much starting material. Prolonged oxygenation gave a very dark solution, which on evaporation gave a black, intractable solid, which defied further analysis.

However, we did receive encouragement from another quarter. In another laboratory within the Department, Mr. John Carnduff had observed that solid samples of 1-isopropyl-2-naphthol (60) were transformed in air overnight to give in high yield a crystalline product having the properties of a hydroperoxide; this, he later showed, possessed the structure (61). He kindly informed us of his findings, and subsequently was able to effect an almost quantitative conversion (60)→(61) *via* photo-oxygenation in benzene solution (3 hr.) This constituted the first preparation of a quinol-

hydroperoxide of a simple naphthol derivative, thereby providing direct support for our proposals.

However the much more tricky obtention of a secondary quinol-hydroperoxide still occupied our attention. Accordingly we attempted the photo-oxygenation of the highly promising analogue, 1-keto-8,9-dihydroxy-1,2,3,4-tetrahydroanthracene (62). To our dismay, the presence of a hydroperoxide was not detected after photo-oxygenation for a month, and the starting material was recovered unchanged.

Tricyclic Systems (Anthranols).

The autoxidation of 9-alkyl and 9-phenylanthranols (63) was first reported by Julian and co-workers, who assigned transannular peroxide structures (64) to the products they obtained.[83] The basis for their assignment rested on an analogy with the photo-sensitised oxygenation of certain aromatic hydrocarbons to give peroxides whose transannular structure had been established by Dufraisse and his school (the discoverers of the reaction).[84] Benzene, naphthalene and phenanthrene do not form such peroxides, but anthracene, naphthacene, pentacene and hexacene

each undergoes this photo-sensitised autoxidation to give a transannular peroxide; a great many derivatives of anthracene and naphthacene behave analogously, e.g. (65), (67), (72). meso-Alkyl, and meso-methoxy groupings greatly facilitate the reaction; for example, the peroxide of 9,10-dimethoxyanthracene (65) forms in a few seconds in sunlight.[85] [This and many other preparations of transannular peroxides, together with their interesting properties, are contained in comprehensive reviews by Dufraisse[84] and Badger.[86]] Despite considerable efforts, Dufraisse was unable to find a catalyst (other than light) which was capable of effecting transannular-peroxide formation. Even at high temperatures, in diphenyl ether as solvent, and with oxygen under pressure, rubrene (66) did not react in the dark. Its solution in benzene did not react in the dark even when kept in contact with oxygen for seven years, whereas in sunlight and air the characteristically fluorescent solutions of rubrene very quickly decolourised with the production of the transannular peroxide, (67).[84]

Julian's report of an in-the-dark formation of a transannular peroxide thus conflicted with the painstaking and virtually unequivocal findings of

Dufraisse. By way of vindication, the latter author, by repeating Julian's experiments, was able to show that Julian's "peroxides" were in fact hydroperoxides.[72] Thus the dark autoxidation of 9-phenyl-10-anthranol (68) was shown to give 9-hydroperoxy-9-phenyl-10-anthrone, (69). This hydroperoxide (69) was also prepared by the action of hydrogen peroxide on the chloroketone, (70). In addition, the methylation product (73) of the hydroperoxide differed from the transannular peroxide (72) which could be obtained by photo-catalysed antoxidation of the methyl ether, (71). Furthermore the transannular peroxide (72) underwent hydrolysis to the hydroperoxide (69).

In each of Julian's experiments, the product was thus a tertiary hydroperoxide (74, R = Me, Et,Ph) derived from a meso-substituted anthranol.[72] However, the preparation of the corresponding secondary hydroperoxide - i.e. the hydroperoxide from anthranol itself - was of particular interest in view of an earlier proposal by Backstrom,[87] who claimed that the anti-oxidant properties of anthracene were best explained by "... the autoxidation of anthracene to anthranol, which substance, being autoxidisable, undergoes reaction with oxygen to give a peroxide,

which is slowly decomposed to anthraquinone." Backstrom, in the same paper,[87] proposed a trans-annular structure for this hypothetical peroxide derivable from anthranol, i.e. (64, R = H). Julian later attempted its synthesis; but was unsuccessful,[83] since his usual conditions of autoxidation - addition of Grignard reagent to the corresponding anthranol acetate, acidification in the presence of diethyl ether to give ethereal fluorescent solutions of the anthranol, and subsequent rapid oxygenation of the ethereal solutions - gave, with anthranol acetate (75), a dimeric product, bianthrone (76) in 75% yield. Julian claimed that the reason for the failure was " ... perfectly understandable, since anthrone [sic] possesses an active hydrogen at position 10, and with oxygen yields the dimer"; however "... the formation of dimer does not rule out the possibility of peroxide formation."[83] This rider was buttressed by the observation that "... the mother liquor yielded gummy crystals, which liberated iodine from potassium iodide, m.p. 140° - 155°C."[83] In the light of Dufraisse's hydroperoxide formulation, this latter observation almost certainly constitutes a partial synthesis of 9-hydroperoxyanthrone (74, R = H). Backstrom had also found the

preparation of a pure sample of anthranol (via the acidification of an aqueous solution of sodium anthranolate) to be especially troublesome, owing to "the ready oxidation of the alkaline anthranolate solutions and also the solutions of the free phenol."[87] Thus, despite the most rigorous attempts to avoid autoxidation, the best preparation produced anthranol of only 97% purity, the predominant impurity being anthraquinone.[87]

The existence of 9-hydroperoxy-anthrone (74, R = H) was of fundamental importance in our proposals relating to the biosynthetic intervention of quinol-hydroperoxides, since perhaps the most notable example of naturally-occurring phenols which can undergo a direct introduction of oxygen is provided by the co-occurrence of anthrones and anthraquinones, e.g. emodin anthrone (77) and emodin (78), cf.[88]. Furthermore the preparations, in general, of secondary quinol-hydroperoxides were considered to be more difficult than those of the corresponding tertiary hydroperoxides, since the former necessarily involve the intermediate formation of secondary radicals of phenols, which are known to be less stable than the tertiary radicals which would be

involved in formation of the latter. However, the secondary radical which would be involved in the formation of the hydroperoxide of anthranol is stabilised to a considerable extent (relative to those involved in other alkyl hydroperoxide-formations) by the two α-phenyl substituents, and it was therefore anticipated that, of all secondary hydroperoxides proposed by us as intermediates, the hydroperoxide of anthranol would form most easily. These considerations lead to the postulate that the biosynthetic formation of the abundantly-occurring[89] anthraquinones involves the direct para-oxygenation of the corresponding anthranols (e.g. 79) to 9-hydroperoxy-anthrones (e.g. 80), which then undergo dehydration to the anthraquinones. This proposed formation of an intermediate quinol-hydroperoxide also explains the occasional reports of the *in vitro* preparation of anthraquinones *via* the autoxidation of alkaline solutions of anthrones, cf. [89].

Although the dehydration of the intermediate hydroperoxide to give anthraquinones obviously occurs in a very great number of cases, there has recently been a suggestion by Money[90] that a plausible rearrangement reaction of the same intermediate hydroperoxide

is able to provide a convincing rationale of the biosynthesis of certain "anomalous" phenolic compounds. The "anomalous" nature of these phenols - which are all "acetate-derived" - refers in particular to their carbon skeletons, which superficially preclude their derivation from "acetate" via a linear polyketide chain. Indeed, Birch[91] and Thomas[92] have each postulated that many of these "anomalous" phenols, for example sulochrin (83) and pinselic acid (84), do in fact derive from branched polyketide chains. An alternative biogenetic pathway was later proposed[93] which involved an unelaborated "oxidative cleavage" of well known hydroxyanthraquinones. Thus the structure of sulochrin and pinselic acid could be derived by application of this process to the anthraquinones emodin (83) and helminthosporin (85) respectively. These ideas are supported by the co-occurrence of emodin-5-methyl ether and sulochrin.[94] The proposal of Money[90] envisages this oxidative cleavage occurring at an earlier stage in the biosynthetic pathway. Thus in the case of sulochrin(82) the hydroperoxide of emodin anthrone (81), which is formed by the usual cyclisation of a linear polyketide chain and subsequent para-oxygenation, is envisaged to rearrange

by the acceptable[54,67,95] mechanism shown to the sulochrin structure. In an exactly analogous manner pinselic acid (84) is considered to derive from the hydroperoxide of helminthosporin anthrone (86) by rearrangement followed by cyclisation to produce the xanthone structure. The biosynthesis of citromycetin (87) and fulvic acid (88) - two other "anomalous" phenols - has similarly been explained by the rearrangement of the hydroperoxides of naphthol derivatives - which, again, were derivable by the usual cyclisation of a linear polyketide chain and subsequent para-oxygenation.[90,92]

In view of the fundamental importance of the existence of 9-hydroperoxyanthrone (74, R = H) in our proposals, we were anxious to effect, if possible, its synthesis. Some features of the earlier observations relating to the autoxidation of anthranol were of considerable bearing on our own projected autoxidation. The first was the obtention of a dimeric compound, bianthrone, in the attempted synthesis of Julian.[83] The significance of this product rests on its almost certain production by the dimerisation of the hoped-for anthranol radical. Although the concentration of the anthranol solutions

which Julian had autoxidised were not disclosed, he did report that the work-up of the reaction-mixture had involved "concentration" of the autoxidised ethereal solution, which procedure "... yielded white crystals, m.p. 230° - 240° (bianthrone)."[83] It appeared, therefore, that Julian would have effected autoxidation in the desired sense (which, as previously mentioned, he was able to do in part) but for his use of insufficiently-dilute anthranol solutions. In view of this, we considered that by the use of very dilute solutions during the autoxidation step, the method of Julian would provide a profitable route to 9-hydroperoxyanthrone. However in preference to this method, which involved the preliminary preparation of anthranol acetate, we decided to prepare anthranol in a direct manner from the readily-available anthrone, as described by Backstrom.[87] We found Backstrom's method of preparation of anthranol most convenient, and were able, by the acidification in the presence of benzene (1 l./1g. anthrone) of a solution of sodium anthranolate at 5°C, to obtain a very dilute benzene solution of anthranol. This solution exhibited the characteristic, blue fluorescence of anthracene derivatives. After washing with water, the solution

was filtered through a pad of anhydrous sodium sulphate. A rapid stream of oxygen was then passed through the solution, resulting in an observable diminution of the fluorescence after only five minutes. After one hour the solution was virtually non-fluorescent, and the oxygenation was discontinued. The benzene was removed at 30°C *in vacuo* to give a pale yellow solid which gave a positive ferrous thiocyanate reaction[96] (see later) indicating the presence of a hydroperoxide. A positive test was also obtained by allowing a sample of the partially (5 min.) autoxidised solution to air-dry on filter paper. Initially we had considered the use of other solvents in which to effect the autoxidation, but, in practice, found benzene most suitable. Diethyl ether was used in the autoxidation experiments of Julian,[83] but in view of the well known autoxidation of ethers to hydroperoxides[70] (laboratory-reagent diethyl ether gives a positive hydroperoxide test), we chose to employ a solvent which was stable to autoxidation, i.e. benzene, carbon disulphide or pyridine. In this way, a positive hydroperoxide test obtained in an autoxidation experiment became uniquely diagnostic of a substrate-hydroperoxide.

Dufraisse[84] had found carbon disulphide especially convenient for the preparation of his transannular peroxides, and we too effected ready autoxidation of anthranol in this solvent; however, it possessed no advantage over benzene. Pyridine was also employed, but in the basic environment the hydroperoxide readily underwent dehydration and only anthraquinone was isolated.

Subsequent ultraviolet spectroscopic analysis of the crude product obtained from the autoxidation in benzene demonstrated the presence of anthrone and anthraquinone as well as 9-hydroperoxy-anthrone. (For details of the ultraviolet absorption of these compounds see below).

The first-tried method of isolation of the pure hydroperoxide involved standard fractional crystallisation of the crude mixture using solvent combinations of chloroform, ethyl acetate, benzene and petroleum ether; none was markedly successful. Chromatography over silica in benzene was attempted, but resulted in ready dehydration of the hydroperoxide to anthraquinone. This dehydration reaction of the hydroperoxide - understandably facile in view of the driving-force of the formation of the highly stable anthra-

quinone - plagued many of the attempted isolation procedures. Even dissolution in methanol effected a slow dehydration and this necessitated recording ultraviolet spectra in this solvent as quickly as possible. However, at room temperature (the hydroperoxide was thermally-labile) and in non-polar solvents the hydroperoxide was stable enough to permit its isolation and characterisation; this was effected in the following manner. The crude product was triturated in benzene at room temperature; this process removed all of the benzene-soluble anthrone and some of the hydroperoxide. The insoluble residue was treated with ethyl acetate at room temperature and filtered. The pale yellow, crystalline residue was anthraquinone. The colourless filtrate contained no anthraquinone, and on cooling to -20°C deposited crystals of the hydroperoxide. A sample, when recrystallised from ethyl acetate at -20°C, analysed correctly for 9-hydroperoxy-anthrone ($C_{14}H_{10}O_3$). The ultraviolet spectrum was recorded rapidly in methanol, λ_{max} = 252 and 272 mμ (ε 30,000 and 15,000), and was very similar to that[72] of 9-hydroperoxy-9-methylanthrone (74, R = Me). In

the presence of base the spectrum of anthraquinone was observed: λ_{max} = 252, 272 and 325 mu (ε 45,200, 14,300 and 4,640). Conversion to anthraquinone was also observed to be complete after 5 hr. in methanolic 0.01N hydrochloric acid solution. The infrared spectrum showed ν_{CHCl_3} = 3520(OH) and 1671cm^{-1}, the latter comparing favourably with the carbonyl absorption frequency of 9-hydroxyanthrone (1676cm^{-1}) and differing significantly from those of anthrone (1653cm^{-1})[98] and anthraquinone (1681cm^{-1}).[98]

At this point a drastic simplification of the preparation of 9-hydroperoxyanthrone was achieved by the observation that anthrone in dilute benzene solution (1mg./ml.) appeared to isomerise to anthranol on exposure to light of wavelength 365mμ; if the solution was simultaneously oxygenated a positive hydroperoxide test was observable after 30 minutes. A blank experiment in which a benzene solution of anthrone was oxygenated under similar circumstances in the dark for 4 days, yielded no hydroperoxide. These findings explain the observation of Schonberg and Mustafa[99] who isolated bianthrone after exposing a concentrated benzene solution of anthrone (120mg./ml.) to air and sun-

light for a week (in Cairo). Here again the transient formation of anthranol *via* photochemical isomerisation followed by free radical formation and dimerisation plausibly explains their result. Although the isolation of the hydroperoxide from our photochemical experiment was not effected owing to lack of time, the potential usefulness of this simple procedure, which by-passes the troublesome anthranol preparation, is obvious.

At this stage there seemed a possibility that we might be able to observe the acid-catalysed rearrangement of 9-hydroperoxyanthrone in the sense that Money[90] envisaged in his proposals relating to the biosynthesis of certain "anomalous" phenols. The products of such a rearrangement of 9-hydroperoxyanthrone (89) would be the hemi-acetal, (90), or its hydrolysis product the hydroxybenzophenone (91). However, despite many attempts, no product other than anthraquinone could be characterised. Treatment of the hydroperoxide in acetic acid with a trace of perchloric acid (cf.[95]) resulted, after 24 hr., in the deposition of pale yellow crystals of anthraquinone. The mother liquors of the reaction mixture (negative hydroperoxide test) were

analysed chromatographically (thin-plate and column) and this showed anthraquinone to be the sole product. Glacial acetic acid alone also effected quantitative dehydration, but more slowly (2-3 days). In methanolic 0.01N hydrochloric acid, the dehydration was complete in 5 hr., as shown spectroscopically.

Nevertheless, the failure of the rearrangement in the case of 9-hydroperoxyanthrone did not, by any means, invalidate the biosynthetic proposals of Money,[90] for those proposals especially related to hydroxyanthraquinones. Chrysazin, 1,8-dihydroxy-anthraquinone (92) is commercially available, and we considered that by reducing it to the corresponding 1,8-dihydroxyanthrone (93), followed by autoxidation, we could obtain its corresponding hydroperoxy-derivative. A study of the possible rearrangement of this compound would then provide a close analogy to the envisaged [90] *in vivo* rearrangements. However, although 1,8-dihydroxy-anthrone exhibited in the presence of base a characteristic, green fluorescence indicative of the presence of the corresponding anthranol, attempts to isolate the anthranol by acidifying

basic solutions in the presence of benzene at low temperature, resulted in virtually complete recovery of 1,8-dihydroxyanthrone, as indicated by the absence of fluorescence in the benzene solution. Some of the orange-red 1,8-dihydroxyanthraquinone, which had resulted from autoxidation of the original basic solution, was also present, as evidenced by its colour. Despite the visible absence of fluorescence, the benzene solution was oxygenated, but without any result. It would thus seem that, in the case of the 1,8-dihydroxy-derivative, the anthrone structure is so stable that even in neutral solution there is virtually no equilibrium with the anthranol structure. The possibilities of bilateral hydrogen-bonding would obviously enhance the stability of the 1,8-dihydroxyanthrone structure (cf. the strong hydrogen-bonding in 8-hydroxytetralones[100]). However the isolation of methyl ethers of hydroxy-anthraquinones[94] may point the way to the characterisation of the true intermediates involved in the *in vivo* oxygenation-rearrangement sequence, and studies on the analogous *in vitro* rearrangements of the hydroperoxides of the corresponding mono- and di-

methoxyanthrones (95, R = H, Me) would accordingly be informatory. Application of the latterly-discovered photo-oxygenation technique to the preparation of these hydroperoxides would, if operable, facilitate such studies.

Tetracyclic Systems (Tetracyclines).

An important feature of the biosynthesis of the tetracyclines is the secondary modification of the largely "acetate-derived" main skeleton, by which chloro, methyl, and hydroxyl groupings are introduced at appropriate methylene groupings of the polyketide chain (see above). The stages in the biosynthesis at which these various introductions occur have not as yet been specified, although the observed accumulation of deschloro- and desmethyl-tetracyclines when thiocyanate and sulphonamide respectively are separately added to normal tetracycline-producing fermentation liquors, has led to the realisation that both chlorination (at C_7) and methylation (at C_6) are modifications which most probably occur at "late" stages cf.[15]. On the other hand, little is known concerning the mechanism

of the biological introduction of the hydroxyl functions at the 5, 6 and 12a positions. Laboratory analogies for 12a-hydroxylation have, nonetheless, been achieved in which the corresponding deoxytetracyclines were hydroxylated chemically, (see above), and also microbiologically by several organisms (Curvularia and Botrytis species);[101] however, the inability of Streptomyces strains to effect this transformation has proscribed consideration of the 12a-deoxytetracyclines as true in vivo intermediates.[101] The mechanism of hydroxylation at positions 5 and 6 has so far not been investigated.

The isolation[38] of 7-chloro-5a,11a-dehydrotetracycline (97) from a mutant strain of Streptomyces aureofaciens, as mentioned previously, constituted the first, reported example of a naturally-occurring p-quinol derivative. Subsequently, the demonstration[39] that this metabolite (97), when added to normal strains of S. aureofaciens, was convertible to 7-chlorotetracycline (99, R = Cl) straightforwardly served to establish its intermediacy in the biosynthesis of 7-chlorotetracycline. Moreover, since this biological transformation involved merely the stereo-

specific addition of hydrogen to an olefinic double bond, it was further suggested that (97) could well be considered as the immediate biosynthetic precurser of chlorotetracycline.[39] The nature of the biological progenitor of 7-chloro-5a,11a-dehydrotetracyclines (97) was of especial interest in view of the novel p-quinol system present in the molecule. Birch, noting that the intermediacy of this chlorodehydro-compound fitted in well with his "acetate" derivation of the main skeleton, has recently proposed that the double bond in this compound marks the site of an aldol ring-closure reaction which generates the B/C ring junction, thus implying that 6-hydroxylation occurs without involvement of an aromatic ring C.[44] However, in the light of our generalised proposals relating to the in vivo intervention of quinols and quinol-hydroperoxides, we were straightway led to propose that the biosynthetic progenitor of the chlorodehydro compound (97), would be the corresponding naphthol derivative, 5a,6-anhydro-7-chlorotetracycline(96); enzymic oxidation of (96) could produce (97) directly or, most probably, involve para-oxygenation to give the intermediate 6-hydroperoxide, (98). Thus the biological introduction

of oxygen at C_6 is postulated to occur at the penultimate stage in the biosynthetic pathway to 7-chlorotetracycline. Analogously, a similar oxygenation is envisaged to occur in the biosynthesis of the other naturally-occurring tetracyclines, 5-hydroxytetracycline, the 6-demethyltetracyclines and tetracycline itself.

It was apparent that these considerations of the mechanism of the biosynthetic 6-hydroxylation might also point the way to a means of completing the total synthesis of the tetracyclines, for one of the major problems encountered during their synthesis had been, and continued to be, the stereospecific introduction of a tertiary hydroxyl grouping at C_6. The benzylic, as well as tertiary, nature of this 6-hydroxyl grouping and the presence of a hydrogen atom disposed trans with respect to it at the neighbouring carbon atom (C_{5a}), made the grouping especially labile towards even mild dehydrating reagents. The nature of the product of the dehydration, a 5a,6-anhydrotetracycline, further explained the facility of this elimination; ring C became aromatic during the reaction, and this provided an obvious driving force for the conversion

of the β-hydroxytetralone system of tetracycline to a naphthalenoid derivative. Here, then, was a grouping which was labile to particularly mild acid treatment and whose introduction in any synthetic route involving acidic reagents had therefore necessarily to be a near-ultimate one. As an alternative to this, its introduction could conceivably have been effected early in a synthetic route, and its presence preserved during subsequent acidic reactions by a protecting group - a method widely used[102] in the synthesis of labile compounds. However, the particularly labile nature of the 6-hydroxyl grouping of the tetracyclines precluded consideration of this procedure, since the usual conditions for introducing appropriate protective groupings provoked the facile elimination referred to above.

At the commencement of our own work (January 1961), no proposals as to the possible mode of introduction of this labile grouping had been published, and most synthetic approaches had accordingly been directed towards either the 5a,6-anhydrotetracyclines or the 6-deoxytetracyclines, both of which lacked the 6-hydroxyl grouping.

The successful attainment of each of these objectives was reported by two groups in 1959 (cf. Introduction). As a result, only two features of the tetracycline molecule remained to be elaborated: the hydroxyl at C_6 and the dimethylamino grouping at C_4. The latter feature had received considerable attention during the studies of Shemyakin on monocyclic model compounds (cf.[15]) and it seemed probable that the introduction could, if desired, be effected at one of the earlier stages of the reported syntheses. This has now (August 1962) been achieved (cf. Introduction).

The main aim of our work was concerned with the possible achievement of an oxidation which would constitute a close *in vitro* simulation of our postulated *in vivo* transformation (96)→(97). Our first thoughts on this topic led us to consider the straightforward application of the standard methods of *p*-quinol preparation to the rather special case of anhydrochlorotetracycline (96). Of the methods available, C-acetoxylation *via* lead tetraacetate/acetic acid offered the most promise and accordingly our preliminary explora-

tory experiments were carried out with this reagent - in the hope of obtaining a 6-acetoxyl analogue of (97). However, the well known[103] versatility of lead tetraacetate as an oxidising agent appeared prejudicial to our aim of effecting specific attack of the aromatic C-ring, and we therefore adopted a circumspect approach. Accordingly we carried out an initial experiment on dedimethylamino-12a-deoxy-anhydrochlorotetracycline (102), a derivative of anhydrochlorotetracycline from which two labile groupings had been reductively removed. For the examination of reaction products we planned to use ultraviolet spectroscopic analysis. The anhydrotetracyclines possess a characteristic maximum at λ = ca. 430mu (ε 8,000), and this high-wavelength absorption can be attributed mainly to the presence of the naphtholic D and C rings. The hoped-for 6-acetoxylation of the aromatic ring-C would disrupt this extended chromophore of the anhydrotetracycline and thereby produce a marked change in the absorption characteristics of the long wavelength region of the spectrum. There was but one example of a compound possessing such a "disrupted chromophore" and this was the naturally-

occurring chlorodehydro-compound (97). It possessed (inter alia) λ_{max} = 375-385mu (ε 4,300); by analogy, we expected the corresponding 6-acetoxyl compounds to possess a similar absorption, and thereby be immediately recognisable.

Treatment of (102) with lead tetraacetate (~~2-5~~ 2.5 equiv.) in acetic acid at room temperature resulted in the uptake of 1 equiv. of lead tetraacetate in 1 hr., 2 equiv. in 2 hr., and 2.35 equiv. in 20 hr. The main product, a monoacetate of molecular formula $C_{22}H_{18}O_8NCl$ indeed showed properties consistent with the desired 6-acetoxylation. Thus its ultraviolet spectrum showed maxima at λ_{max} = 262 and 392mu (ε, 28,700 and 4,920), and the infrared spectrum, ν_{CHCl_3} = 1758 (acetate), 1699 (C = O), 1644 (amide), and 1567cm^{-1} (amide), was also consistent; we therefore adopted the hoped-for 6-acetoxy derivative, (103), as a working structure. Although the ultraviolet spectrum of this product, by analogy with that of the chlorodehydro-compound (97), was consistent with a $\Delta^{5,11a}$ assignment of the newly-generated olefinic double bond, the non-conjugated ketonic carbonyl absorption at ν = 1699cm^{-1} was better explained by a $\Delta^{5,5a}$ assignment. These seemingly

conflicting observations could, however, be plausibly explained by invoking a solvent-dependent tautomerisation of the complex conjugated β-diketone system present in the molecule; we accordingly assigned a tautomeric structure, (103). α-Hydroxy derivatives of ketones are reducible by zinc/acetic acid to the parent ketones;[104] we anticipated that similar treatment of (103) would effect reductive removal of the vinylogously situated 6-acetoxyl grouping (cf. part structure, 104) resulting in the regeneration of the anhydro-starting material, (103). Gratifyingly, we were able to effect this zinc-induced transformation by observing the regeneration of the characteristic ultraviolet absorption maximum (λ = 448 mμ) of (102).

At this juncture, a paper appeared[105] describing the preparation of a mono-acetate *via* the lead tetra-acetate oxidation of the 10-methyl ether of dedimethylamino-12-deoxyanhydrochlorotetracycline, (105). Surprisingly, the ultraviolet spectrum of this product, λ_{max}= 265, 331 and 386 mμ (ε, 24,700, 13,300 and 26,400), differed markedly (even allowing for the presence of the 10-methoxyl substituent) from that of our own monoacetate - especially in respect of the high

ε = 26,400 of the long wavelength absorption maximum (λ = 386 mμ). The author of the paper, Muxfeldt, had set out to effect introduction of an acetoxyl grouping at the 12a position, but since the spectral data of the monoacetate he isolated was inconsistent with such an assignment, he tentatively suggested a 2-acetoxyl structure. We were suspicious of this tentative 2-acetoxyl assignment, and accordingly methylated our monoacetate with diazomethane in an attempt to correlate the two products. The carbon (but not the hydrogen) analysis of the amorphous methylation-product we obtained was consistent with a monomethylether-monoacetate assignment, as were the spectral data, λ_{max}= 268 and 367 mμ (ε 22,000 and 3,300), ν_{CHCl_3} = 1759 (acetate), 1694 (C=O and 1644cm^{-1} (amide); however this product differed from Muxfeldt's monoacetate. A comparison of the ultraviolet spectrum of Muxfeldt's starting-material (105), λ_{max}= 265, 320 and 380-386 mμ (ε 25,800, 12,300 and 26,200), with that of his monoacetate convinced us that he had not effected, as we had, a disruption of the naphtholic ring system, for the two spectra were virtually superposable. The retention of the intact B-C-D

chromophore was further supported by the infrared spectrum (ν_{CHCl_3} = 1745 (acetate) and 1610cm^{-1} (C = O)) of Muxfeldt's monoacetate, the absorption maximum ν = 1610cm^{-1} being characteristically attributable to the strongly hydrogen-bonded C_{11}-carbonyl function of the anhydrotetracyclines (e.g. (102) has ν = 1619cm^{-1}). Once Muxfeldt had unequivocally eliminated the 12a assignment for the introduced acetoxyl his allocation of the grouping to the 2-position did not seem implausible. Nonetheless, we still were not convinced of its correctness; on the other hand we were not, at the time, able to suggest an alternative structure. However, since we were contemplating further oxidation studies on anhydrochlorotetracycline, we decided to abandon further investigation of Muxfeldt's "anomalous" monoacetate in the hope that we would be able to reconsider the problem at a later date. As it transpired, the later studies shed no new light on the problem; however a recent reappraisal of Muxfeldt's and our own data has enabled us to assign the benzyl acetate structure, (106), to Muxfeldt's anomalous acetate. The spectral data accorded well with this structure; in particular, the new assignment of a benzyl acetate to the ν = 1745cm^{-1} is

immediately plausible. There appear to be two feasible modes of formation of the benzyl acetate. The first involves the direct acetoxylation of an intermediate mesomeric benzyl radical (109), and the second, a rearrangement under acidic conditions of the initially-formed 6-acetoxy derivative, (107), *via* the intermediate quino-methine, (108); the analogous rearrangement of (49)⟶(50) (q.v.) is also envisaged to occur *via* a quino-methine.[75] Unfortunately, owing to lack of time, we have not been able to carry out any confirmatory rearrangement studies on our own 6-acetoxy-compound, but such studies would, we feel, lead to final clarification of this problem.

Encouraged by our preliminary success, we turned to the study of the lead tetraacetate oxidation of anhydrochlorotetracycline itself, (96). Initially we were interested in the extent of oxidation which could be produced in the presence of excess reagent. We therefore added gradually 4 equiv. of lead tetraacetate to a solution of anhydrochlorotetracycline (96) in acetic acid, and by removing aliquots for iodometric titration were able to follow the uptake of lead tetraacetate. The first drop of lead-

tetraacetate turned the originally orange solution dark green, and after further additions the colouration intensified rendering the solution opaque. This made iodometric titration a precarious procedure, but after 30 minutes estimation showed an uptake of ca. 3.3 equiv. The product was a brown, intractable solid, having λ_{max} = 365-370 mμ (ε ca. 5,500). The spectrum of this product was encouraging, and the oxidation was therefore repeated on a larger scale with 2.5 equiv. lead tetraacetate in the hope of securing a characterisable product. Trituration of the dark product with boiling chloroform gave a near-black, semi-solid gum, which was rejected since its ultraviolet spectrum (λ_{max}= 335 mμ) was not indicative of the desired 6-acetoxy derivative. The brown chloroform-insoluble residue was suspected to be a lead complex, which, it was thought, might yield further chloroform-soluble material on acidification. Treatment of the brown residue with methanolic hydrochloric acid in the presence of chloroform did indeed yield a reddish organic layer, which yielded on evaporation a brown amorphous solid. Although we were not able to crystallise this product, nor obtain a sample for analysis, we have assigned to

it the hoped-for 6-acetoxy structure, (110), on the basis of its spectral data, λ_{max} = 265-270 and 388-394 mu (ε 13,800 and 4,200); ν_{CHCl_3} = 1741 (acetate), 1702 (C = O) and 1643cm^{-1} (amide).

In the meanwhile, however, we had been considering the alternative methods of p-quinol preparation which involved direct hydroxylation, i.e. the employment of peracids such as Caro's acid or peracetic acid. We felt that if these were found to be applicable to the case of anhydrochlorotetracycline, then we should have no difficulty in characterising the reaction-product - since direct hydroxylation would yield the well-characterised naturally-occurring chlorodehydro-compound. It would, moreover, provide us with a direct *in vitro* analogy for our postulated *in vivo* hydroxylation. We therefore embarked on a series of experiments using Caro's acid. The awkwardness of this reagent is well known, and consequently there are little or no experimental details in the literature other than the original work of Bamberger carried out at the beginning of this century. The phenols which Bamberger[53] was able to hydroxylate were all partially water-soluble, and consequently he had

comparatively little difficulty in obtaining his desired products. However anhydrochlorotetracycline is virtually water-insoluble, and as a result our main problem was one of achieving a "predominantly homogeneous" reaction-mixture. Our preliminary attempts to patterr a profitable experimental procedure on the Bamberger technique were totally unsuccessful. Bamberger had found[53] that Caro's acid in the presence of excess magnesium carbonate was capable of effectively hydroxylating, for example, p-cresol, (23) (q.v.); he termed this technique oxidation with "neutral" Caro's Acid. When we tried this technique - with the addition of methanol to enhance the solubility of the anhydrochloro-compound - we observed no sign of any oxidation product. We repeated the experiment without the addition of magnesium carbonate in 0.2N sulphuric acid. The substrate dissolved but little, even on vigorous shaking, but the very small amount of substrate which dissolved did appear to react with the Caro's acid; filtration of the undissolved starting-material gave a filtrate showing λ_{max} = 370 - 380 mu, at once indicative of 6-hydroxylation. The yield of this product, however, was abysmally low (< 2mg.). We

considered a better reaction medium might be one using aqueous acetic acid, and by this means we were able to work with a homogeneous reaction-mixture. The addition over a period of 2 days of 2 equiv. of Caro's acid produced the hoped-for change in the ultraviolet spectrum, and we were able to observe the advent of the characteristic λ_{max} = 375 - 385 mu of the chlorodehydro-compound (97).

The clumsiness and poor results attained by these attempted tetraacetate and peracid oxidations led us to consider a more elegant approach involving direct use of molecular oxygen; as will be seen this approach was entirely successful.

The oxygenation of a naphthol derivative had not hitherto been reported; the well-characterised anthrone hydroperoxides (44), (q.v.), which had been prepared *via* the oxygenation of anthranol derivatives,[72] provided us with the clue that the analogous naphtholic derivatives might similarly undergo oxygenation to give quinol-hydroperoxides. In order to test this hypothesis we attempted initially the direct oxygenation of a benzene solution of anhydrochlorotetracycline (96). We had at our disposal two methods of identification of the hoped-for 6-hydroperoxy-

derivative; as well as the well-tried ultraviolet spectroscopic method of analysis, we were aware that the presence of a hydroperoxy grouping in an organic compound could conveniently be detected by means of a characteristic spot-test. Several such spot-tests were listed in a compendium devoted to the preparation and properties of the organic peroxides,[70] and we found the ferrous thiocyanate test-reaction[96] to be one of the most convenient. The basis of this test depends on the oxidation of the colourless ferrous thiocyanate anion to the characteristically blood-red ferric thiocyanate anion. We were thus able to follow the course of oxygenation experiments by the periodic spot testing of aliquots (samples were allowed to air-dry on filter paper). Percolation of oxygen for 14 days through a benzene solution of anhydrochlorotetracycline (96) produced no evidence, by the above two criteria, for the production of any hydroperoxide.

However, the successful application of photo-catalysed autoxidation to aromatic hydrocarbons (Dufraisse[84]) and to olefins (Schenck[106]) prompted us to attempt an analogous photo-catalysed oxygenation of anhydrochlorotetracycline. Although not strictly

comparable, the observed[107] photo-catalysed oxygenation of α-terpinene, (111), to give the transannular peroxide, ascaridole, (112), was of more than incidental interest, since ascaridole is unique in being the only naturally-occurring organic peroxide so far recorded. The above laboratory synthesis provides an eminently feasible in vitro analogy for the biological formation of ascaridole; further, since α-terpinene gives a polymeric peroxide on autoxidation in the dark, we felt that the use of photo-catalysed oxygenation in the case of anhydrochlorotetracycline would constitute a closer analogy to an in vivo environment. As light source we chose to employ two 20 watt fluorescent lamps, and these were disposed each side, vertically, of a Pyrex glass tube through which oxygen was percolated. Photo-oxygenation in this way of a dilute solution of anhydrochlorotetracycline (96) in benzene for 7 days caused no perceptible change in the ultraviolet spectrum of the reaction mixture, nor was a hydroperoxide test observably positive. However concentration of the solution drew attention to the formation of a small quantity of a sparingly soluble product whose yellow colour was in marked contrast

to that of the starting anhydrochlorotetracycline (deep orange). Most promisingly this material did indeed give a positive hydroperoxide test. A larger-scale procedure was therefore devised for the photo-oxygenation of anhydrochlorotetracycline in 1g. batches, using a more efficiently-directed light source. Under these conditions the yellow product slowly precipitated from solution after 5 days and coated the walls of the vessel. Recycling furnished an overall yield of 70% of crude hydroperoxide. Latterly the acquisition of a Hanovia Photochemical Reactor greatly facilitated the photo-oxygenation studies. The immersion lamp of 450 watts, which constituted the main feature of the apparatus, proved an outstandingly dramatic improvement on the original light source; photo-oxygenations which had previously required 8-10 days could now be accomplished in 6 hours. Filtration and crystallisation from chloroform of the crude hydroperoxide gave golden yellow needles all of whose properties were fully compatible with its formulation as 7-chloro-6-deoxy-6-hydroperoxy-5,5a-dehydrotetracycline (98). Thus it gave a strong hydroperoxide test and its elementary analysis fitted the molecular formula

$C_{22}H_{21}N_2O_9Cl$. The ultraviolet spectrum, λ_{max} = 249 and 375-385 mu (ε 24,100 and 4,510) was almost superposable on that of 7-chloro-5a(11),11a-dehydrotetracycline (97), λ_{max} = 250 and 385-395 mu (ε 21,800 and 5,060), but the presence in the infrared spectrum of ν = 1707cm^{-1} (non conjugated ketone) was better explained by a 5,5a assignment of the double bond. Those tetracyclines which possess an intact, enolisable 10,11,12-trioxygenated chromophore exhibit a characteristic bathochromic shift in the long wavelength band of the ultraviolet spectrum in the presence of Ni^{2+} ion;[108] the presence of this feature in (98) was confirmed by observing the expected[108] shift, λ_{max} (Ni^{2+}) = 410-415 mu (ε 11,000).

The specific reduction of the 6-hydroperoxy-derivative (98) to the corresponding 6-hydroxy-derivative (97) was our next objective, and we planned to effect this transformation by catalytic hydrogenation. Initially we were interested in the possible extent of reduction. We anticipated an uptake of three molecular proportions of hydrogen, since there was present in the molecule an olefinic double bond and a hydrogenolysable chlorine atom, in addition to the hydroperoxy grouping. Hydro-

genation of the 6-hydroperoxy-derivative (98) in methanol-triethylamine resulted in absorption of 2.9 mols. of hydrogen to give a product which exhibited the spectral characteristics of tetracycline (99, R = H), the expected[109] product. It was significant however that the uptake of the initial mol. of hydrogen was extremely rapid. It was suspected that this initial rapid reduction had involved a selective attack on the readily-reducible hydroperoxy grouping. We therefore repeated the reduction without the triethylamine and stopped the reaction after the uptake of one molecular proportion of hydrogen (150 seconds). The yellow product was crystallised from diethyl ether to give yellow needles; it did not give a hydroperoxide test, and its ultraviolet spectrum did not markedly differ from that of the starting-material. The spectrum, (λ_{max} = 254-256 and 385-390 mμ (ε 23,800 and 5,220); λ_{max} (Ni^{2+}) = 411-416 mμ (ε 12,000)) of the product was identical with that of the naturally-occurring 7-chloro-5$\overset{\wedge}{\wedge}$,11$\overset{\wedge}{\wedge}$-dehydro compound, (97), but again the ν = 1709cm^{-1} in the infrared spectrum was more consistent with a Δ5,5a assignment of the double bond. In the paper[38] describing the isolation

of the naturally-occurring chlorodehydro-compound, an infrared maximum at ν = 1724cm^{-1} was attributed to the C_{11} carbonyl; however, this determination was carried out on a non-crystalline derivative. In order to confirm unequivocally that our synthetic product was identical with the compound of natural provenance, we converted an authentic sample of the hydrochloride of the naturally-occurring compound to the crystalline free base, and compared our synthetic compound with it. The ultraviolet spectra were superposable, as were the two infrared spectra (KBr disc). Furthermore, the optical rotations confirmed that the stereochemistry of the synthetic product was identical with that of the natural compound i.e.

	synthetic (97)	natural (97)
$[\alpha]_D$	(0.4% in $CHCl_3$) + 210°	(0.38% in $CHCl_3$) +212°
$[\alpha]_D$	(0.51% in 0.03NHCl) + 15°	(0.65% in 0.03NHCl) +15.5°

This constituted a striking *in vitro* analogy for our *in vivo* proposals. The appropriate radiochemical incorporation studies, which, we anticipated,[110] would provide the desired confirmation of the intermediacy of 5a,6-anhydro-7-chlorotetracycline (96) in the biosynthetic pathway, were subsequently reported,[111]

e.g. ^{36}Cl-5a,6-anhydro-7-chlorotetracycline (96), when fed to Streptomyces aureofaciens, yielded ^{36}Cl-7-chlorotetracycline (99, R = Cl).[111] Furthermore, since the biosynthesis of each of the other naturally-occurring tetracyclines is considered to involve an analogous 6-hydroperoxidation, the in vitro transformation (96)—>(98)—>(97)—>(99) offers mutatis mutandis a solution to the total stereospecific synthesis of all of the tetracycline antibiotics.

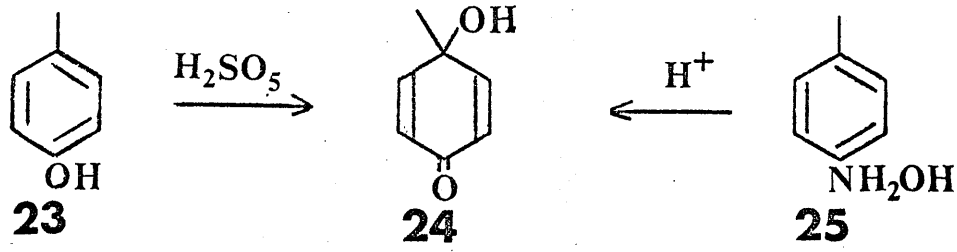
In order to attempt to verify the proposed applicability of this reaction sequence to other anhydrotetracyclines, we have photo-oxygenated anhydrotetracycline (96, H for Cl), anhydro-6-demethyl-7-chlorotetracycline (100) and anhydro-5-hydroxytetracycline (101). In the case of anhydrotetracycline (96, H for Cl) we have been able to establish the presence of a hydroperoxide in the photo-oxygenated reaction mixture, but have been unable to effect its separation from unchanged starting material. Anhydro-6-demethyl-7-chlorotetracycline (100) did not yield a hydroperoxide even after photo-oxygenation for 20 hours employing a 450 watt lamp; the brown solid which during this time gradually coated the sides of the vessel exhibited an ultraviolet spectrum similar to that

of starting material. On the other hand, the photo-oxygenation of anhydro-5-hydroxytetracycline (101) produced a wholly intractable product which neither showed a positive hydroperoxide test, nor a characterisable ultraviolet spectrum, nor an infrared spectrum in which any maxima were uniquely discernible. The failure to effect hydroperoxide formation in the case of the 6-demethyl analogue (100) was presumably due to the relative instability of the secondary radical necessarily involved in its formation; the failure to effect hydroperoxide formation in the case of the simple model naphthols, (56) and (62) supports this hypothesis - especially as the preparation of the tertiary hydroperoxide (61) of a simple naphthol derivative (60) was so easily effected (q.v.). The failure in the case of anhydro-5-hydroxytetracycline (101) may be attributed to the characteristic lability which the presence of the 5-hydroxyl grouping confers on the molecule, cf.[4].

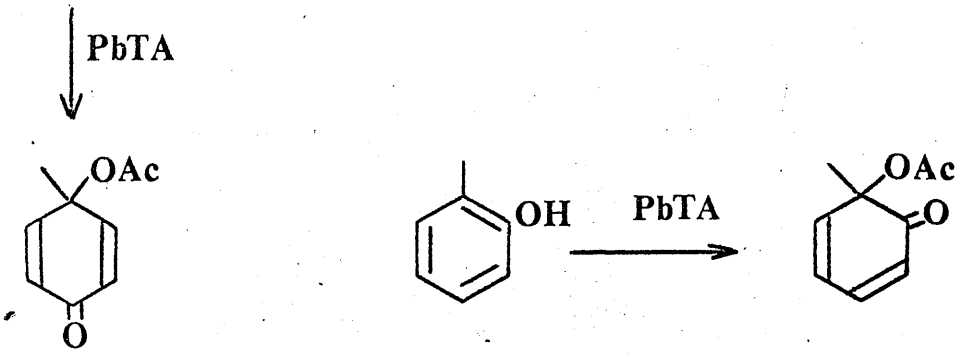
The employment of a photo-sensitiser often facilitates a photochemical transformation and frequently provides a means of inducing a reaction which may not otherwise occur (cf.[106]).

The preparation of the elusive secondary hydroperoxide of the 6-demethyl compound (100) will, we consider, most probably be achieved by the use of a suitable photo-sensitiser. (The photo-oxygenation of anhydro-7-chlorotetracycline (96) in the presence of 1,2- or 3,4-benzpyrene has been observed to produce an enhanced yield of hydroperoxide (98) in a shorter reaction time[112]).

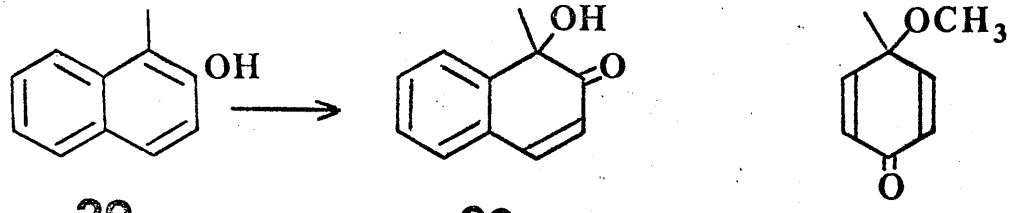
Meanwhile a research group[113] in the United States is, we understand, at present nearing the completion of the first total stereospecific synthesis of a naturally-occurring tetracycline via a route which involves the ultimate photo-oxygenation of an anhydrotetracycline.



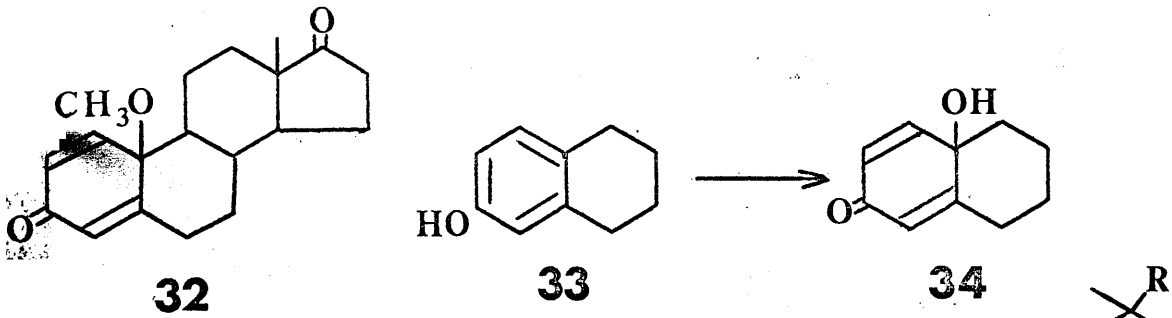
23 24 25



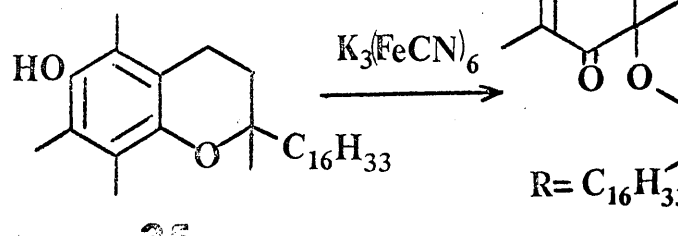
26 27 28



29 30 31



32 33 34



35 36

Chart 1

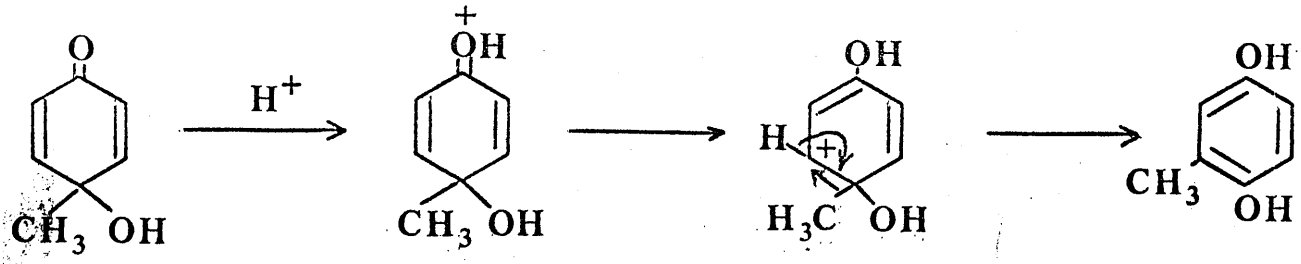


Chart 2

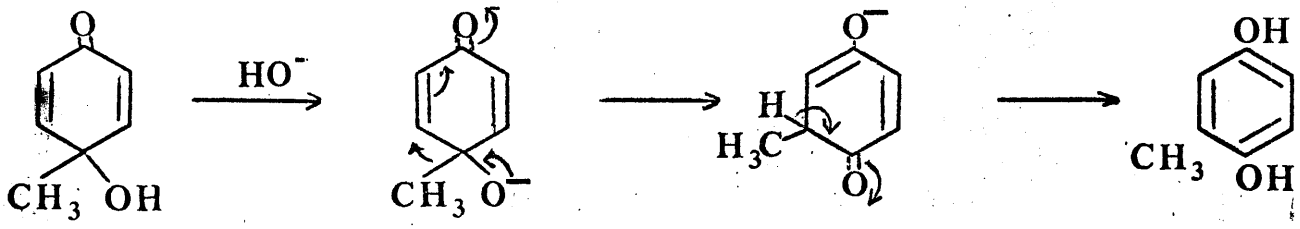
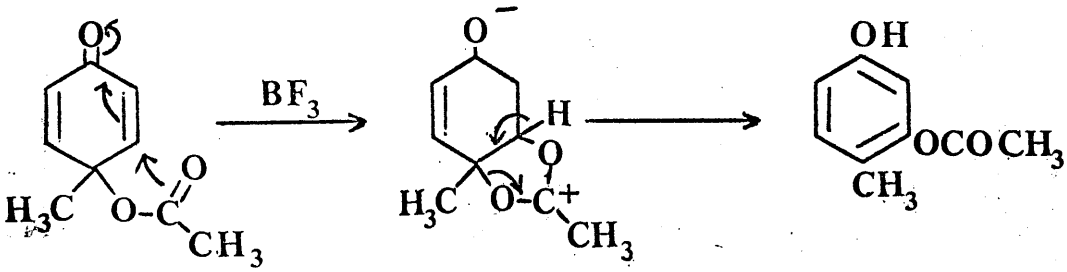
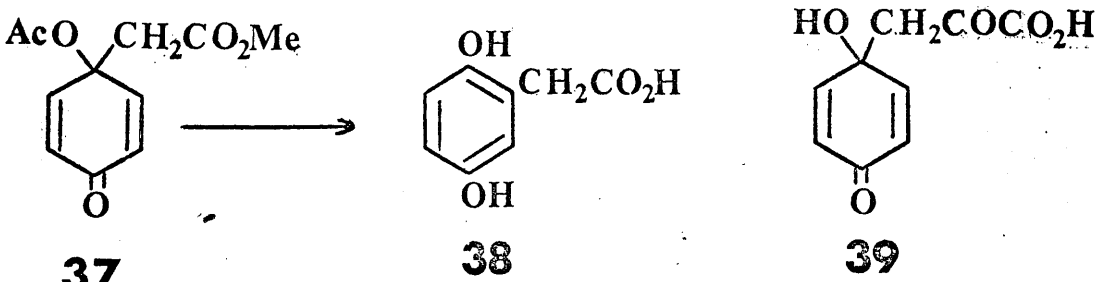


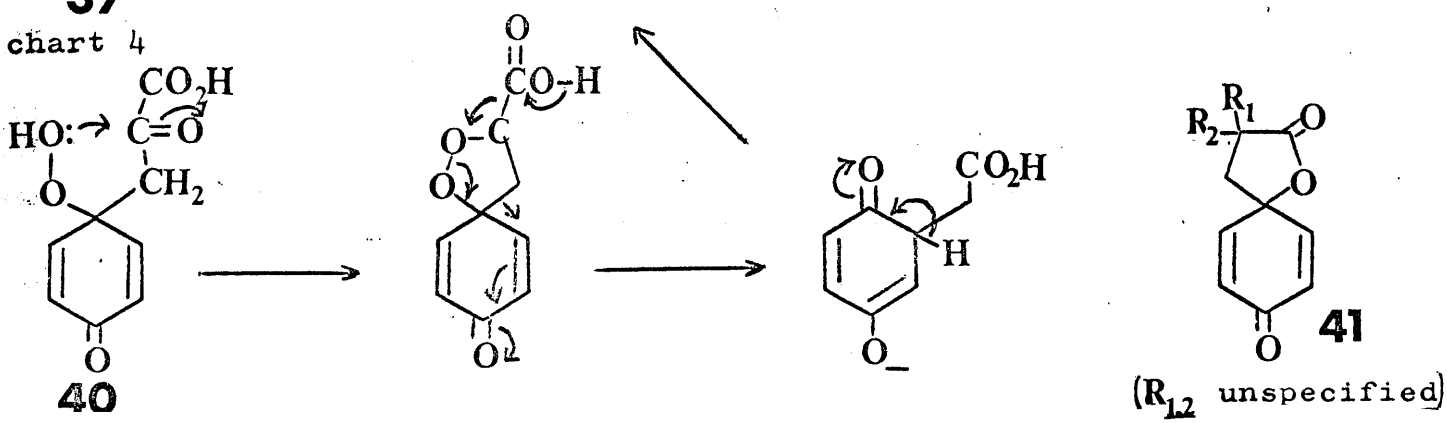
Chart 3





37 38 39

chart 4



40 41 ($R_{1,2}$ unspecified)

Chart 5

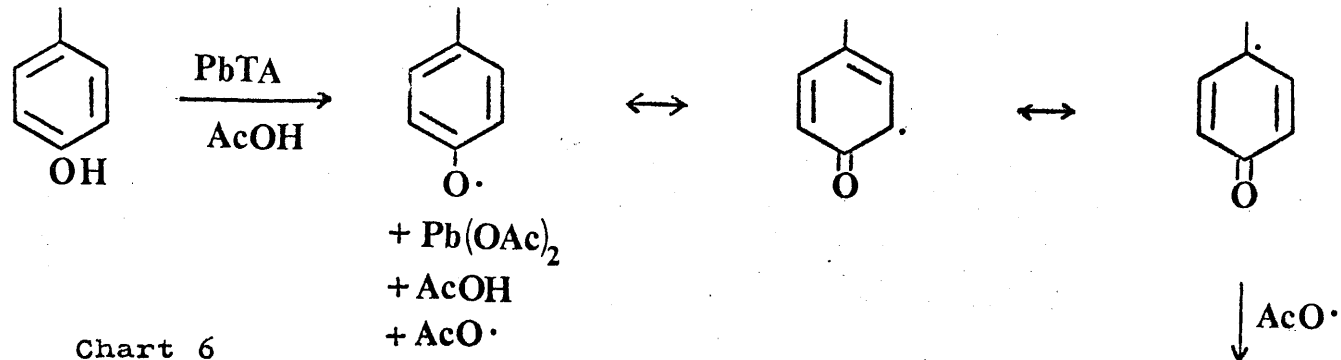
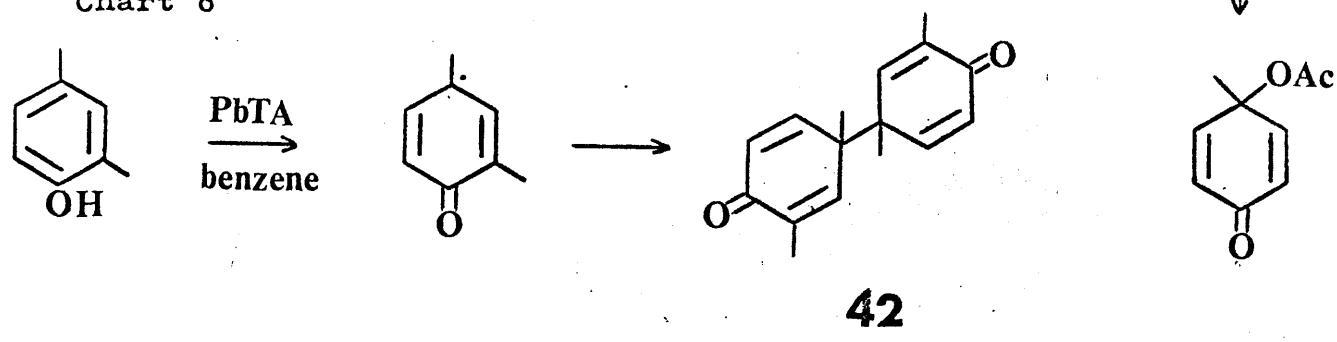
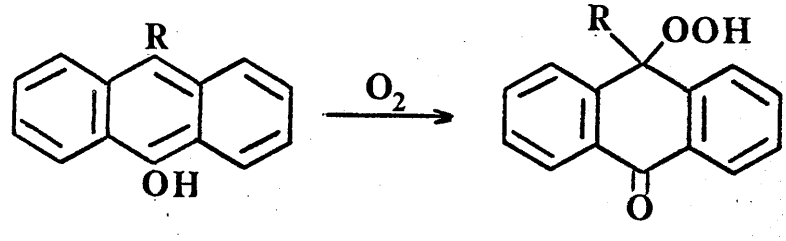


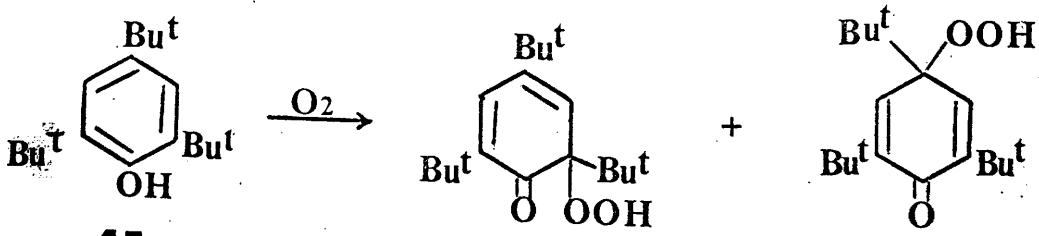
Chart 6



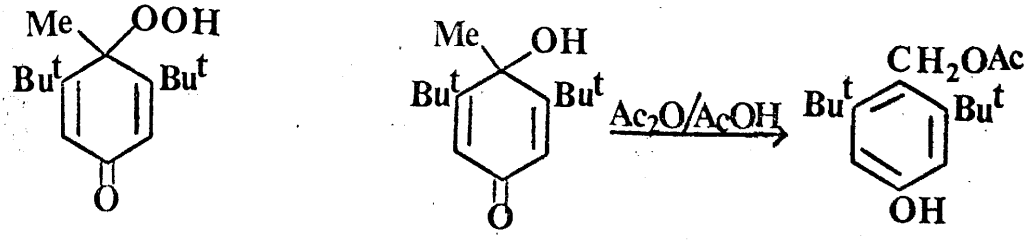
42



43 44

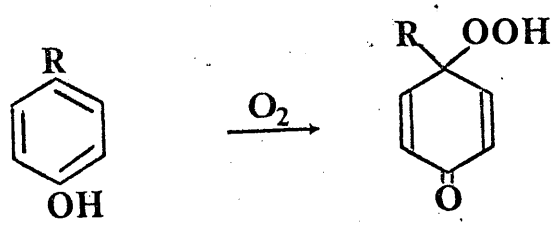


45 46 47



48 49 50

Chart 7



51

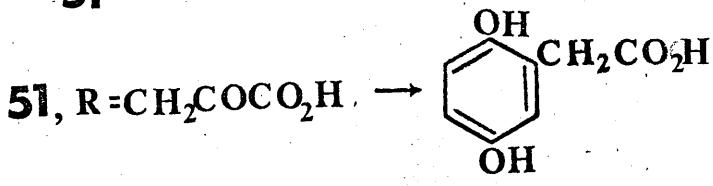
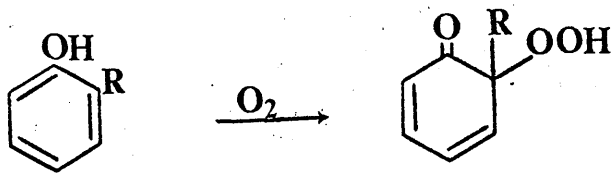
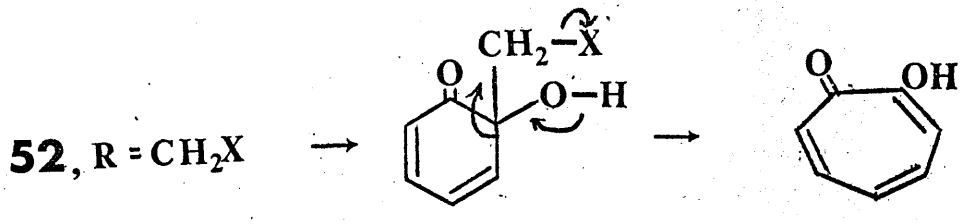


Chart 8



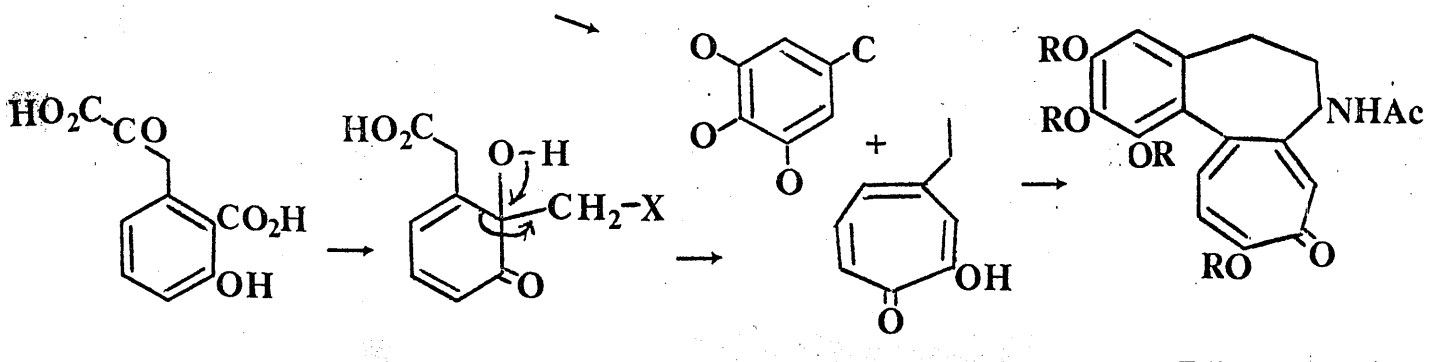
52



53

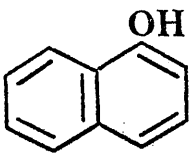
Colchicine Biosynthesis

phenylalanine

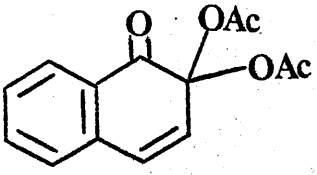


54

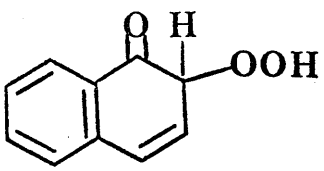
55 (R=CH_3)



56

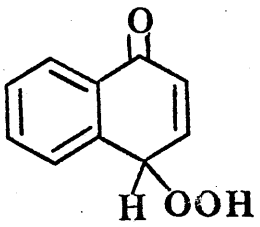


57

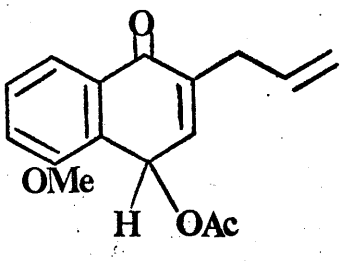


58

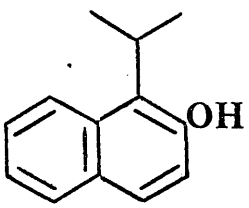
+



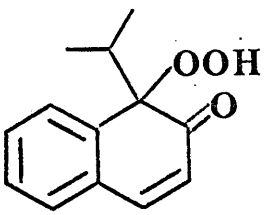
58A



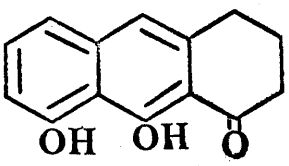
59



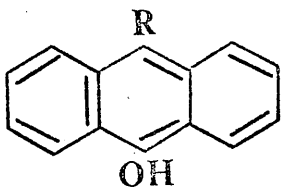
60



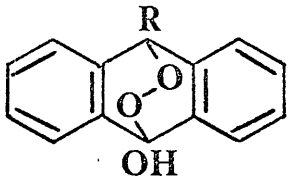
61



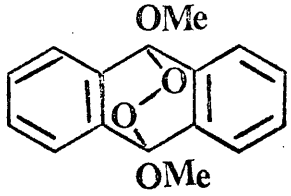
62



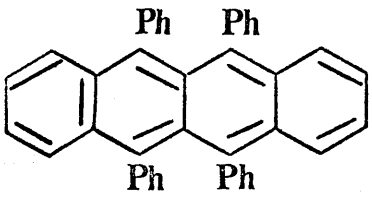
63



64

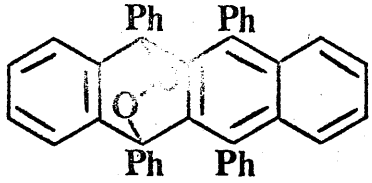


65

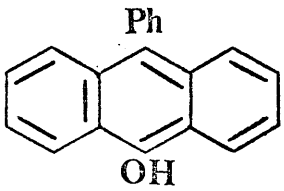


66

O_2 →

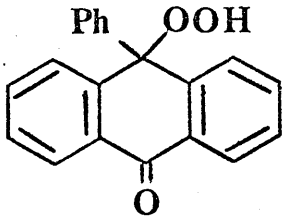


67



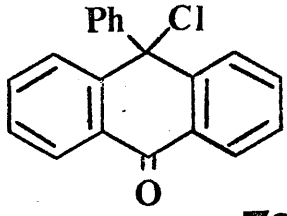
68

O_2 →



69

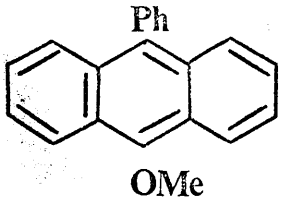
H_2O_2 ←



70

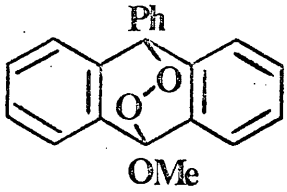
Me_2SO_4 ↘

H_2O ↓

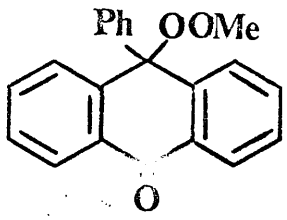


71

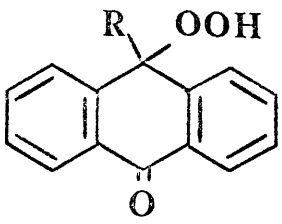
O_2 →



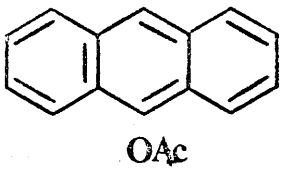
72



73

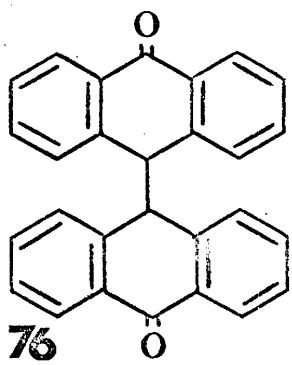


74

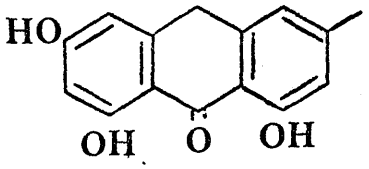


75

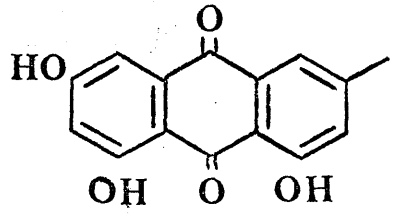
→



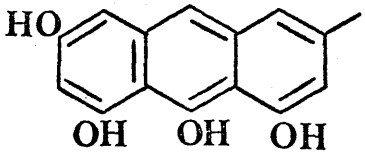
76



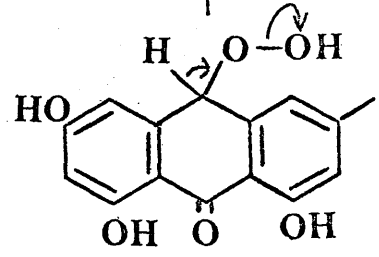
77



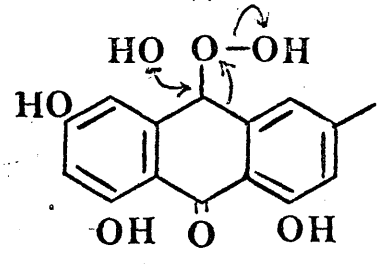
78



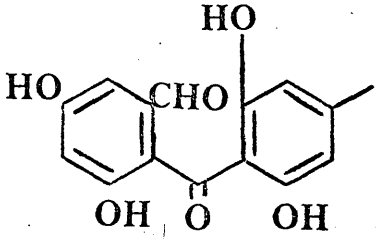
79

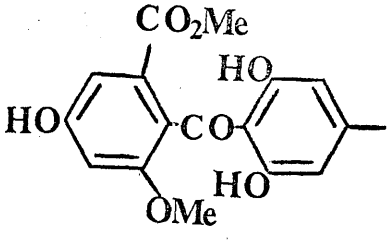


80

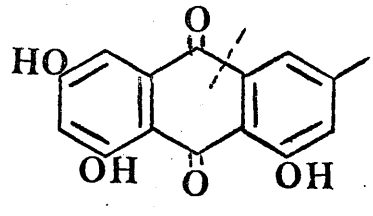


81

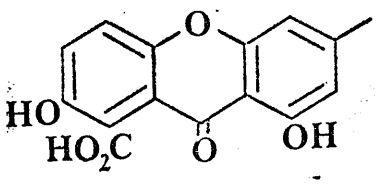




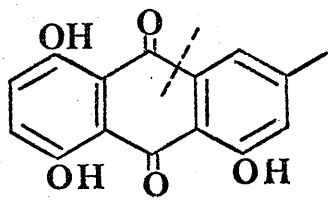
82



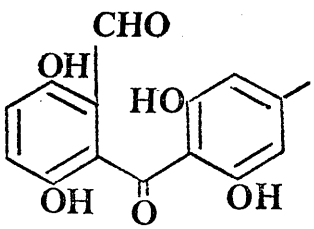
83

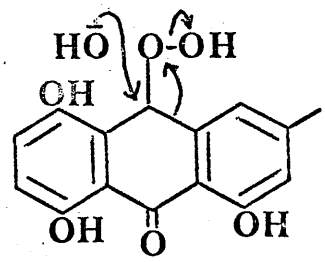

HO
HO2C
O
OH

84

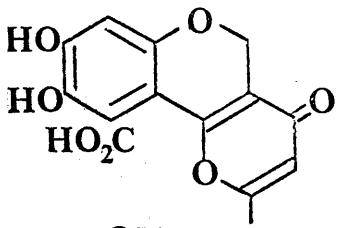

OH
O
OH
O
OH

85

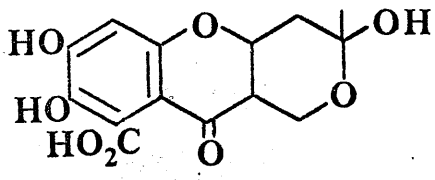

CHO
OH
HO
OH
O
OH


HO
O-OH
OH
OH
O
OH

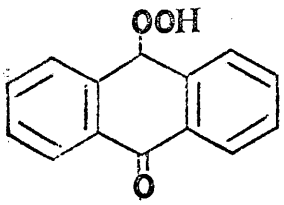
86


HO
HO
HO2C
O
O
O

87

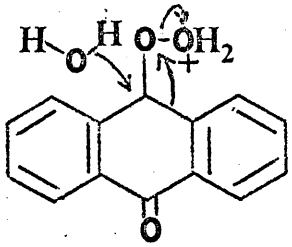

HO
HO
HO2C
O
O
O
OH

88

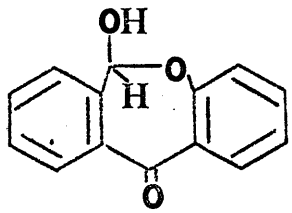


89

$\overset{H^+}{\rightleftharpoons}$

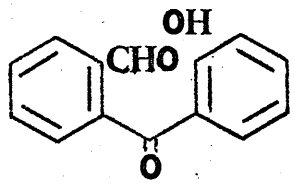


→

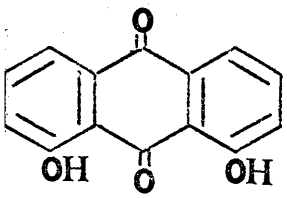


90

↓

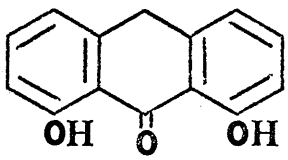


91



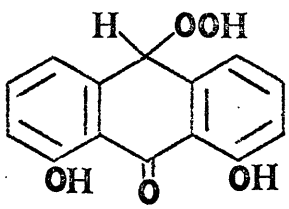
92

→

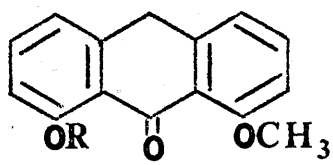


93

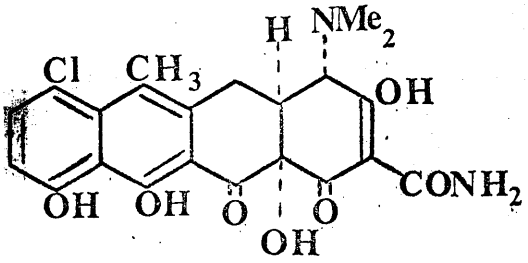
↓



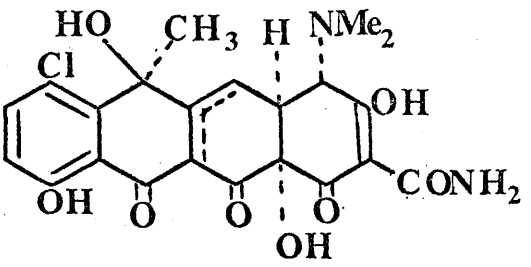
94



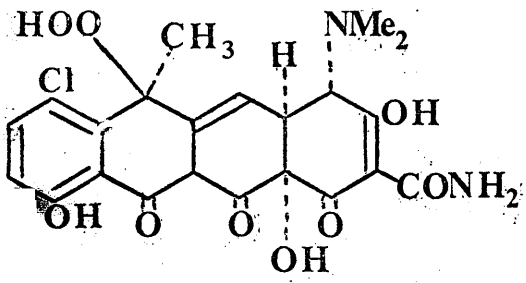
95



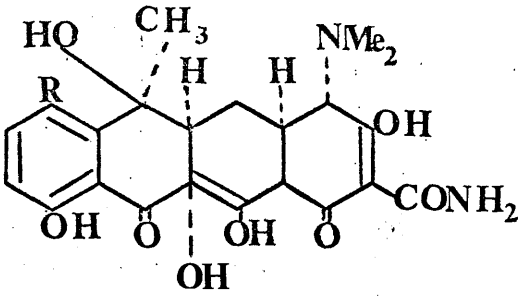
96



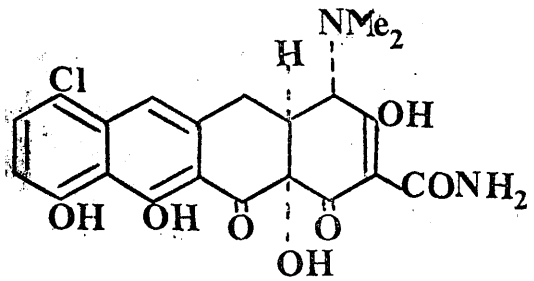
97



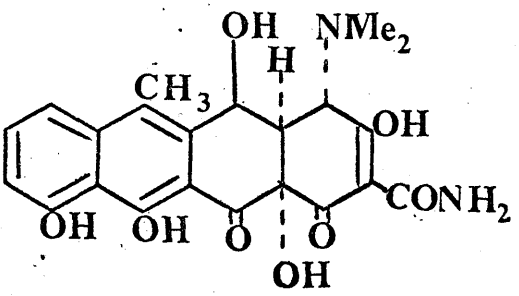
98



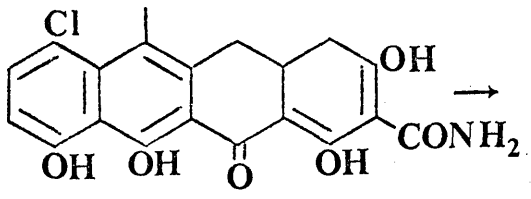
99



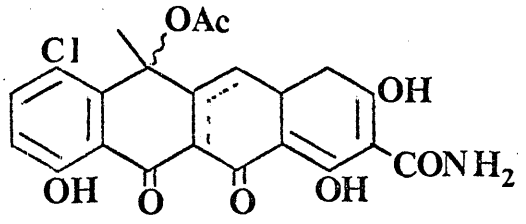
100



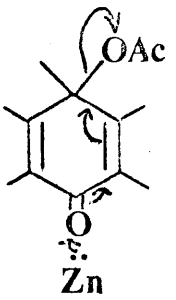
101



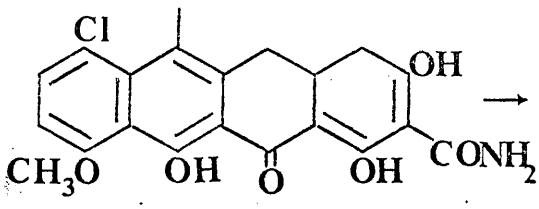
102



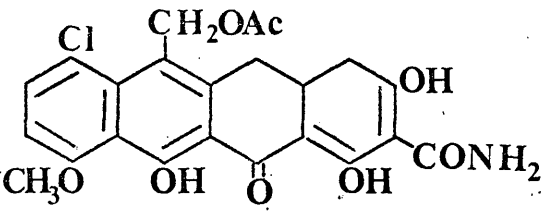
103



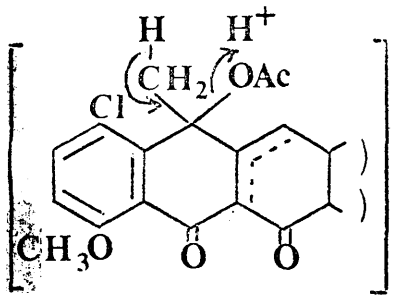
104



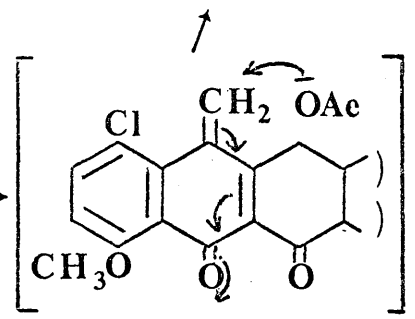
105



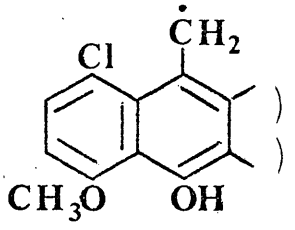
106



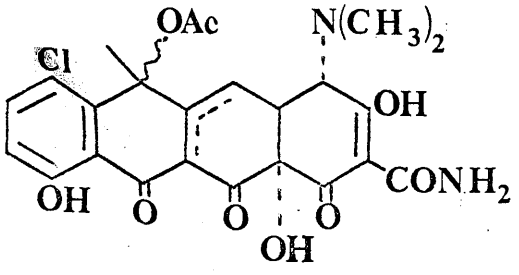
107



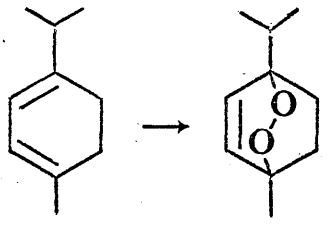
108



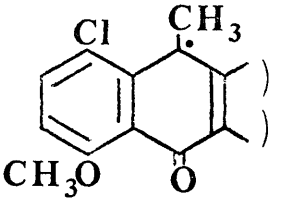
109



110

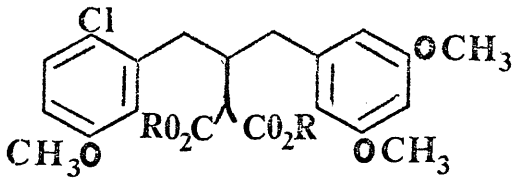


111 112



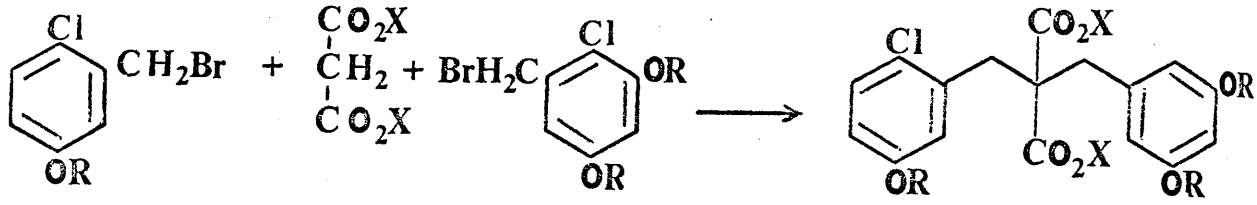
Part IV

Approaches to the Synthesis of Tetralones related to Tetracyclines.

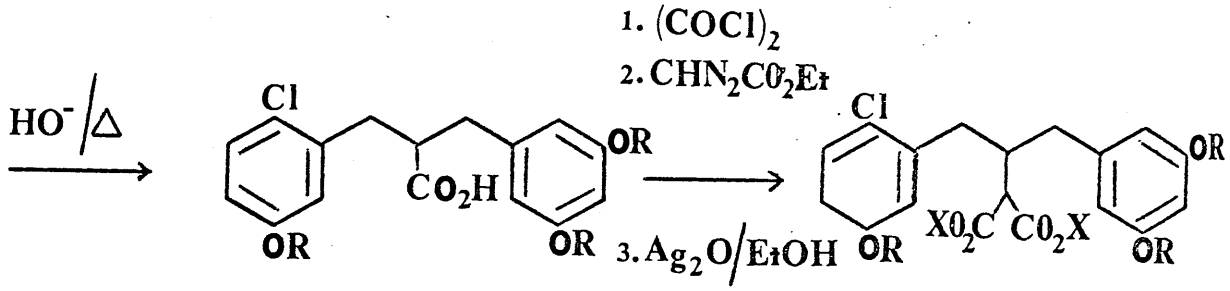


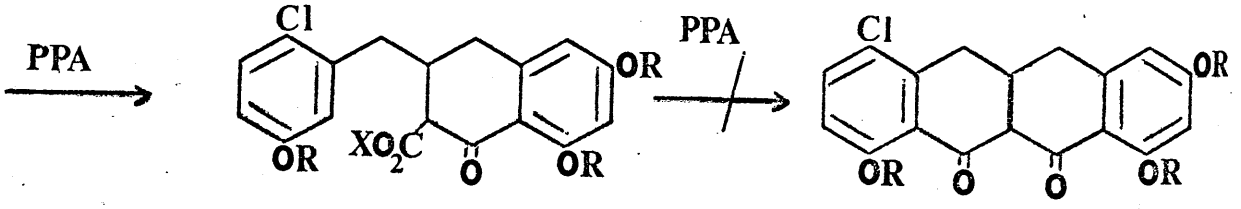
113

Chart 9 $(R=CH_3)$



114 115





116 117

Approaches to the Synthesis of Tetralones related to Tetracyclines.

A novel approach (for a comprehensive review of other approaches cf.[15]) to the synthesis of tetracyclines involves a double cyclisation of malonic ester derivatives such as (113). This would represent a welcome simplification of the problem as the two central B and C rings would thereby be produced concurrently complete with the desired heteroannular β-diketone feature.

Bhati,[118] in a preliminary study of this approach in this department, has prepared (113, R = Et) from two benzenoid fragments (114) and (115), and has been able to convert it into the tricyclic derivative, (116); however he was unable to effect the hoped-for polyphosphoric acid cyclisation of (116) to the tetracyclic derivative (117), [Chart 9]. This failure, plausibly ascribed by Bhati to steric factors, was disappointing but we considered that by directing our attempts towards the synthesis of a slightly modified malonic ester derivative, (126) or (135), we would probably be able to effect the ultimate cyclisation in the desired sense.

As is evident, each of our proposed objectives, (126) and (135), differed from Bhati's malonic ester derivative (113) in respect of the prospective ring-D. The possible amelioration attainable by the modification we had formulated rested on the presence in the prospective D-ring of a 2-carbethoxy substituent (or its feasible progenitor a 2-cyano substituent); this would obviously render the formation of ring C feasible by a Dieckmann type of ring closure. Moreover, since this substituent would provide a uniquely reactive site (C_2) for ring closure, the presence in the same ring of the chlorine atom (used by Bhati[118] as a blocking group) was no longer necessary, and was accordingly dispensed with. Although Bhati's route appeared to be amenable to our modified approach, we chose to formulate and investigate two different approaches, each employing more easily available starting materials; these are portrayed schematically [Charts 10 and 11]. Each of the approaches involved several novel transformations, and these were conveniently investigated by a study of simple and readily-available analogies. Accordingly most of our work has comprised an exploratory study of model compounds; many of the

proposed, novel transformations present in the main approaches have been shown thereby to be feasible synthetic processes. In addition, some progress has been made on one of the main approaches [Chart 11].

Our first series of experiments related to the approach outlined in Chart 10. The synthesis of o-cyano-m-methoxyacetophenone (121) from m-hydroxyacetophenone (118) has been described,[119] and since we expected the condensation under basic conditions of this cyano-compound (121) and 3,5-dimethylbenzaldehyde (122) to proceed in a straightforward manner to give the chalcone (123), we concentrated our attention on the series of steps by which the chalcone (123) is converted to the corresponding dibenzyl ketone (124). As a simple analogy for this series of steps we chose to attempt the transformation using the commercially-available benzalacetophenone (137). A recrystallised sample of (137) was reduced (2 mol. H_2) in ethanol in the presence of palladium-charcoal to give the saturated alcohol (138), which, when distilled in the presence of a trace of p-toluene sulphonic acid, formed trans-1,3-diphenyl-prop-1-ene (139). Oxidation of (139) with perbenzoic acid gave the corresponding

epoxide (140), which on distillation partially (20%) rearranged to dibenzyl ketone (141). Levy[120] has also observed this thermal rearrangement; he[120] was further able to show that the epoxide (140) was susceptible to acid rearrangement to give uniquely the same ketone, dibenzyl ketone (141). We did not attempt to confirm these findings, since similar results had been published in a comprehensive review of the mechanism of epoxide reactions.[121] We were thus able to demonstrate the feasibility of the transformation of the chalcone to the corresponding dibenzyl ketone.

The nature of the malonic ester moiety of both of our synthetic objectives, (126) and (135), differed slightly (a cyano grouping replaced one of the carbethoxy grouping) from that (113, R = Et) used by Bhati.[118] This minor modification was employed since cyanoacetic ester was known to condense with ketones under Doebner conditions much more smoothly and efficiently than diethyl malonate. In each of our approaches the condensation of cyanoacetic ester with a substituted dibenzyl ketone, (124) and (134), was proposed. In order to investigate this reaction, e.g. (124)—>(125), and the subsequent reductive step (125)—>(126), we embarked on a series

of model experiments employing the commercially-available dibenzyl ketone (141). The condensation of dibenzyl ketone (141) with cyanoacetic ester has been reported by Dufraisse *et al.*[122] We were able to repeat this preparation, and obtained the unsaturated cyano-ester (142) in virtually quantitative yield. However all attempts to hydrogenate the unsaturated cyano-ester (142) failed. Since Dufraisse[123] had also reported the condensation of malonitrile with dibenzyl ketone (141) to give the unsaturated dinitrile (142; CN for CO_2Et), we repeated this preparation in the hope of being able to effect reduction of the unsaturated dinitrile to the saturated dinitrile (143; CN for CO_2Et). We obtained a good yield of the unsaturated nitrile (142; CN for CO_2Et), and were able to hydrogenate this product in ethyl acetate in the presence of Adam's catalyst to give the previously unreported saturated dinitrile (143; CN for CO_2Et). An attempt was made to cyclise this compound with concentrated sulphuric acid, but the orange powder which was isolated by filtration after pouring the homogeneous sulphuric acid solution into water, was not the expected tetralone as shown by its

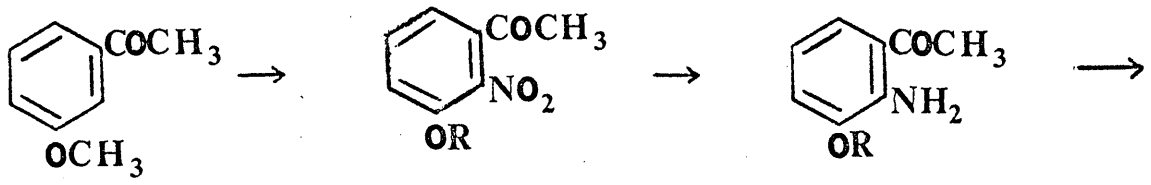
spectral data. This product could not be obtained crystalline, and no further investigation of its constitution was made. The unsuccessful reduction of the unsaturated cyano ester (142) was difficult to explain, since Cope[124] had found many similar compounds to be reducible in a straightforward manner. At this stage the observed[122] thermally-induced ring closure of the unsaturated cyano-ester (142) to the naphthol derivative (144) provided a clue to a convenient means of avoiding the troublesome reductive step - although instead of a tetralone analogue we appeared to be nearing the synthesis of a hydroxynaphthacene analogue (145). Nonetheless, we repeated the thermal cyclisation of (144) and obtained 45% yield of (145). This reaction is most conveniently carried out at 240° in acetamide,[124] and offers a most convenient synthetic method of preparation of these tricyclic derivatives.

Concurrently with this work on model compounds we were amassing the starting materials for the approach outlined in [Chart 11]. The synthesis of 7-methoxyindanone (130) has been described,[125] and involved initially the condensation of acrylonitrile with phenol to give the adduct (127);

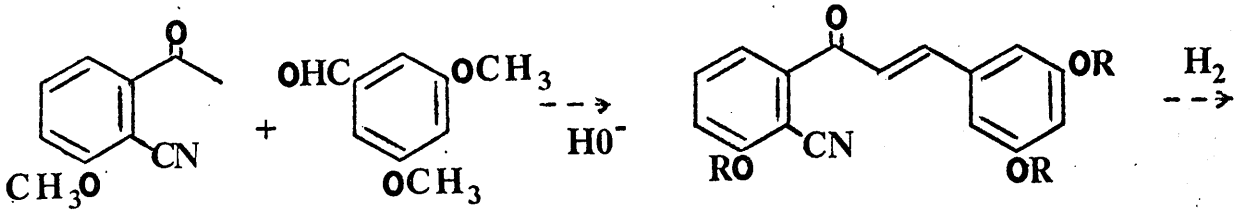
thereafter hydrolysis, and ring closure gave chroman-3-one (128). Rearrangement, in the presence of aluminium chloride at 200°C, gave 7-hydroxyindanone (129), methylation of which gave 7-methoxyindanone (130). 3,5-Dimethoxybenzaldehyde (122) was conveniently prepared from 3,5-dimethoxybenzoic acid *via* the Stevens reaction.[126] The preparation of the 3,5-dimethoxybenzylidene derivative (131) was achieved in good yield, as was the subsequent reduction to give (132). In an analogous experiment the 3,5-dimethoxybenzylidene derivative (131; H for Me) of 7-hydroxyindanone (129) was prepared.

Time has not permitted the continued investigation of these two routes but the above initial results are now being extended by another worker.

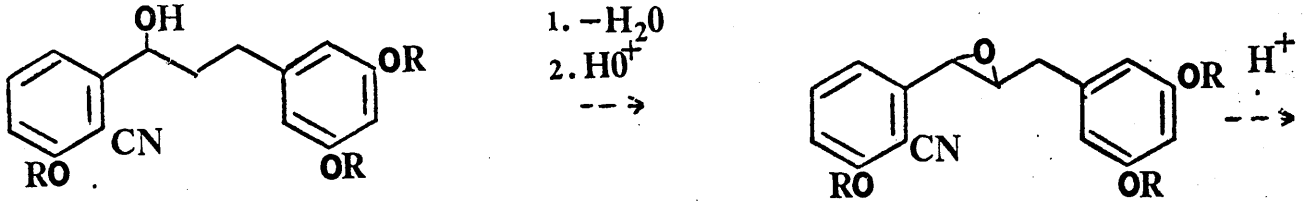
Chart 10 $(R=CH_3)$

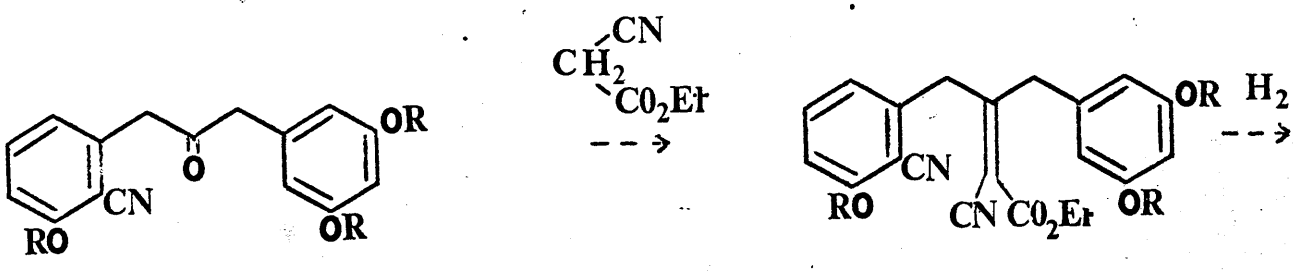


118 119 120

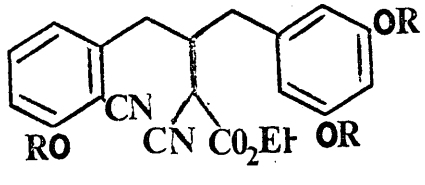


121 122 123



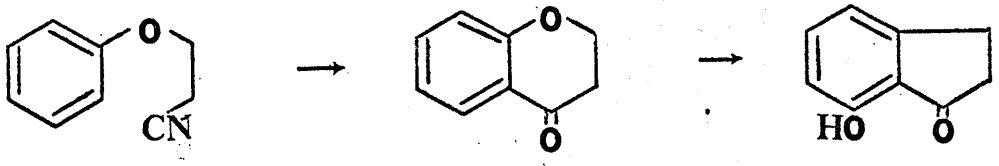


124 125

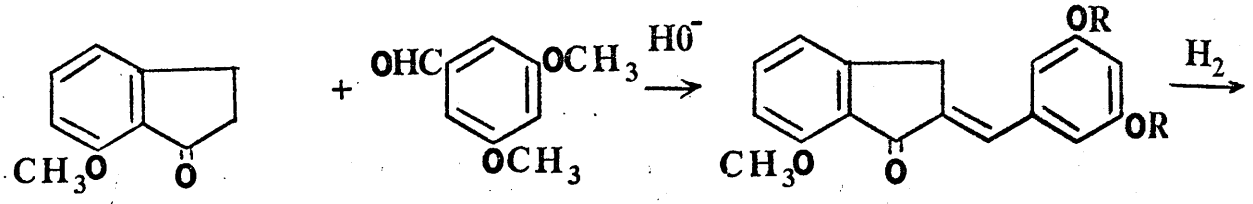


126

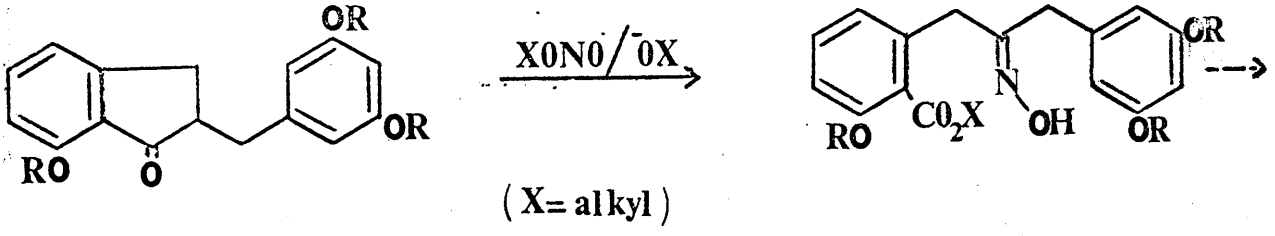
Chart 11 $\left(R=CH_3\right)$



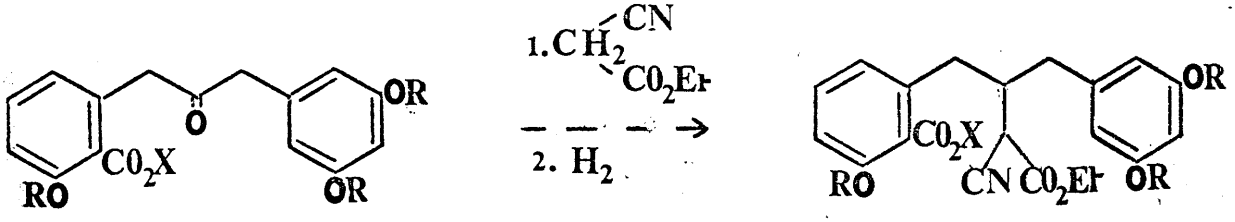
127 128 129



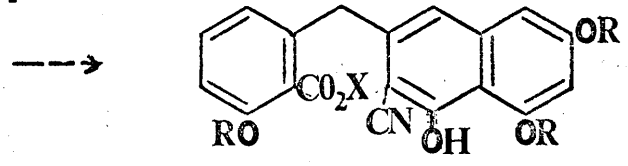
130 122 131



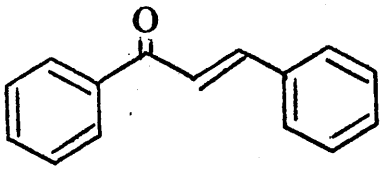
132 133



134 135

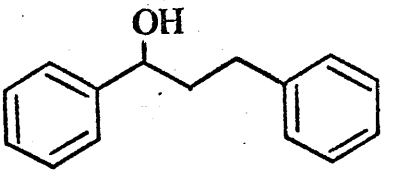


136



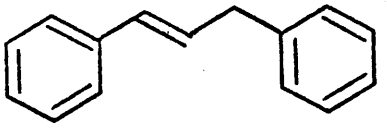
137

H_2 →



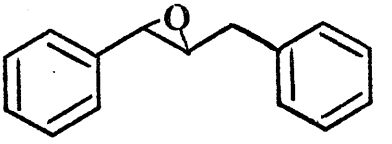
138

H_2O →



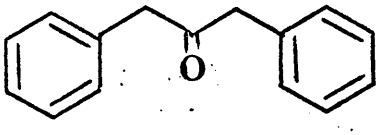
139

HO^+ →



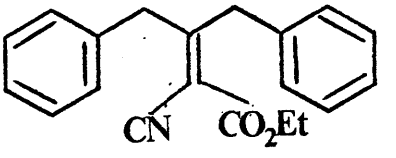
140

H^+ or →, △



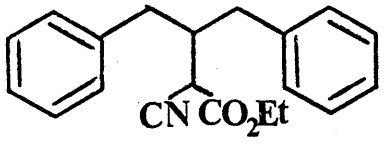
141

$CH_2(CO_2Et)(CN)$ →

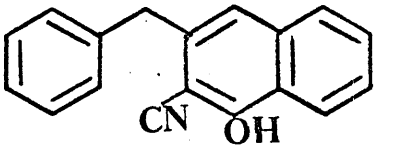


142

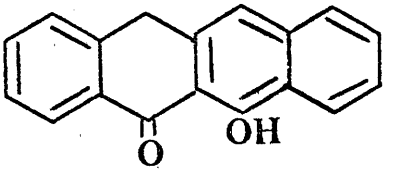
↓ △



143



144



145

Part V

Experimental.

Experimental

Melting points were determined on a Kofler block. In view of the generally non-characteristic nature of the melting or decomposition points of tetracycline derivatives, none were recorded.

Ultraviolet absorption spectra, unless otherwise stated, were measured on a Unicam S.P. 500 spectrophotometer for 0.01N methanolic hydrochloric acid solutions. Absorption maxima recorded in basic solution [λ_{max}(base)] were measured for 0.01N methanolic sodium hydroxide solutions; those recorded in the presence of divalent nickel ion [λ_{max} (Ni^{2+})] for 0.01M methanolic nickel chloride solutions.

Infrared spectra for liquid films and for nujol mulls were taken with a Perkin Elmer Infracord spectrometer; for solutions (chloroform) with a Unicam S.P.200 Spectrometer.

Buffered silica gel was prepared according to the method of Muxfeldt.[133]

The light petroleum used had b.p. 60-80°.

Photo-oxygenation of 1-Naphthol(56)

1g. Scale/4X20W. Lamps (see below)

1-Naphthol(1g.) in benzene(11.) was photo-oxygenated for 21 days. Samples were removed daily to determine infrared spectra. After 5 days a sample showed ν_{nujol}= 1710cm^{-1}, as well as the characteristic 1-naphthol maxima. An ultraviolet spectrum of this product was indistinguishable from that of starting material. After 21 days the solution had darkened somewhat. Removal of solvent gave a black intractable solid which defied further analysis.

Attempted photo-oxygenation of 1-Keto-8,9-dihydroxy-1,2,3,4-tetrahydroanthracene(62)

1g. Scale/4X20W. Lamps (see below)

A dark green sample of (62), m.p.125-127°, was recrystallised from diethyl ether to give ochre needles, m.p.126-127°, which gave a green colouration with methanolic ferric chloride.(Found:C,73.41;H,5.30. $C_{14}H_{12}O_3$ requires C,73.67;H,5.30%), λ_{max}=219,265 and 406mμ (ε 24,750, 45,400 and6,330), ν_{nuj}=3370(OH) and1620cm^{-1}(C=O).

(62)(1g.) in benzene(11.) was photo-oxygenated for one month. The starting material was recovered unchanged.

Anthrone

A yellowish commercial product was triturated at room temperature to yield a petroleum-soluble extract which was free of anthraquinone. Two recrystallisations of this material from ethyl acetate gave colourless needles, m.p.(rapid heating) 152-156°, λ_{max}=252;(base)=272 and 376mμ

9-Hydroperoxyanthrone(74,R=H)

Finely pulverised anthrone(2g.) was added to a gently boiling solution of sodium hydroxide(10g.) in water(100 ml), and the flask plugged with cotton wool. The mixture was simmered for five minutes, during which time most of the anthrone dissolved to give a yellow-orange solution of the sodium salt of the anthranol anion. The solution was rapidly cooled to -5°, and transferred to a separating funnel containing 6N sulphuric acid(60ml) and benzene (21.) held at 0°. The partially frozen benzene rapidly melted with shaking, and quickly gained an intense blue fluorescence. The colourless aqueous layer was run off, and the benzene layer was washed with water and filtered through a pad of anhydrous sodium sulphate.

A rapid stream of oxygen was percolated through the solution, resulting in an observable diminution of the fluorescence after only 5 min.. After 1hr the solution was virtually non-fluorescent, and the oxygenation was discontinued. The benzene was removed at 30° *in vacuo* to

to give a pale yellow solid(2.02g.), which gave a positive hydroperoxide test.

The crude product(2.02g.) - a mixture of anthrone, 9-hydroperoxyanthrone and anthraquinone - was treated with cold benzene(10ml), triturated, and filtered. The filtrate contained most of the anthrone, together with some hydroperoxide(colour test). The remainder of the anthrone was removed by repeating the process using 5ml. of benzene. The insoluble portion(1.6g.) was treated with cold ethyl acetate(10ml), which dissolved all of the hydroperoxide, and left an insoluble pale yellow residue of anthraquinone(0.8g.). The colourless ethyl acetate solution of the hydroperoxide was cooled to -20°, at which temperature the product(0.3g.) crystallised with scratching. A sample was recrystallised from ethyl acetate for analysis.(Found: C,74.33;H,4.46. $C_{14}H_{10}O_3$ requires C,74.23;H,4.50%)
λ_{max}(MeOH)=252 and 272mμ(ε 30,000 and15,000)
λ_{max}(base)=252,272 and 325mμ (ε 45,200,14,300 and 4,640)
ν_{CHCl_3}=3520(OH) and1671cm^{-1}(C=O). A melting-point was not determined in view of the known thermolability of the compound.

Attempted Autoxidation of Anthrone in the Dark

Anthrone(36mg.) was dissolved in benzene(45ml) in a tube which was surrounded with aluminium foil. The solution was maintained at 55-60° during oxygenation. After

6 days the solution was still colourless and did not give a hydroperoxide test.

Photo-oxygenation of Anthrone to 9-Hydroperoxyanthrone

Anthrone(36mg) was dissolved in benzene(45ml) in a quartz tube to give a non-fluorescent solution, and oxygenated during exposure to a Hg-vapour lamp(λ=365mμ). After 0.5hr. the warm(T=ca.50°) solution showed a yellowish tinge, and a sample when removed gave a positive hydroperoxide test. A similar test after 1hr. was very strong. No precipitation had occurred. Lack of time prevented the isolation of the 9-hydroperoxyanthrone.

Attempted Rearrangement of 9-Hydroperoxyanthrone(74,R=H)

9-Hydroperoxyanthrone(100mg) was dissolved in AnalaR acetic acid(10ml), and perchloric acid(1 drop) added. After 3hr, the solution no longer gave a positive hydroperoxide test, and pale yellow crystals of anthraquinone(68mg) had deposited. Analysis of the filtrate by Thin Layer Chromatography(silica gel) showed that anthraquinone was the sole product. In a similar experiment rigorous work-up led to the isolation of anthraquinone(92mg) in quantitative yield.

In the absence of perchloric acid, the dehydration occurred more slowly(2-3 days). Again, anthraquinone was quantitatively isolated.

1,8-Dihydroxyanthrone(93)

The reduction conditions ofNaylor and Gardner[114] were used.

1,8-Dihydroxyanthraquinone(2.4g.,10mM), granular tin (11.8g.,100mM) and acetic acid(150ml) were boiled to give a very dark orange mixture. Concentrated hydrochloric acid(27ml) was added dropwise, while the mixture was maintained at the reflux temperature. When 10ml. of the hydrochloric acid had been added, the solution became pale yellow. After the final addition of acid, the mixture was refluxed for 1 hr., and filtered hot at a glass sinter. On cooling, a yellow crystalline solid deposited, which was isolated by filtration(1.4g.). Recrystallisation from light petroleum gave lemon yellow platelets,m.p.178-180°. λ_{max}(MeOH)=257,288 and 355mμ;(base)=386mμ. Basic solutions of (93) show an intense green fluorescence, which in air rapidly give crimson solutions of the corresponding anthraquinone.

Attempted Preparation of 1,8-Dihydroxy-9-hydroperoxy-anthrone(94)

1,8-Dihydroxyanthrone(100mg) was treated with 2N sodium hydroxide(5ml) to give at first a green fluorescent solution, which rapidly turned crimson. Benzene was added and the mixture neutralised by the addition of sulphuric acid at 0°. The benzene layer did noy become fluorescent, and oxygenation of the benzene layer did not lead to a positive hydroperoxide test.

Dedimethylamino-12a-deoxy-5a,6-anhydro-7-chloro-tetracycline(102)

This compound was prepared from 7-chlorotetracycline hydrochloride by zinc/acetic acid reduction and dehydration as reported by Stephen et al.[115] Its characteristic ultraviolet spectrum confirmed its identity: λ_{max}=274,338,386and448mμ (ε 30,600,4,860,5,050 and8,450). The solution infrared spectrum, hitherto unrecorded, provided additional confirmation: ν_{CHCl_3}= 1642(amide),1619(C=O) and 1568cm^{-1}(amide). Although most usually of unprepossessing red-brown appearance when prepared, samples of this compound were found to be of sufficient purity for further use - the λ_{max} and ε values providing a convenient and rigorous means of characterisation. The use of nitrobenzene as crystallising solvent[115] was found to be wasteful of material and troublesome to manipulate, and was accordingly avoided.

Treatment of (102) with Lead Tetraacetate(2.5 equiv)

(102)(320mg,0.80mM) was dissolved in warm glacial acetic acid(140ml) and briskly stirred during the room temperature addition of freshly-recrystallised lead tetraacetate(875mg,2.5equiv). Thiosulphate titration of aliquots showed uptake of 1 equiv in 1hr., and 2 equiv in 2hr..After 20hr. 2.35 equiv had been consumed and the solution was clear and red in colour. The solvent

was decreased in volume to 30ml. by evaporation under nitrogen at 70°/20mm., and water(200ml) added. Extraction with benzene gave an emulsion which was broken by filtration through celite; this also removed a small amount of dark solid, which was probably an inorganic lead salt. The benzene extracts were dried(Na_2SO_4) and on evaporation gave a dark brown solid(352mg). This was dissolved in 1:1 chloroform-benzene(45ml) and chromatographed over buffered silica gel(10g.) with the following results.

Elutriant			Weight eluted in mg.
1. Chloroform-benzene	(1:1)	200ml	42.2
ditto	(5:4)	50ml	
2. Chloroform		400ml	134.3
3. Chloroform-methanol	(95:5)	50ml	25.0
4. ditto	(1:1)	50ml	15.3
			216.8

Part of fraction 2(100mg) was dissolved in hot benzene, and on cooling a non-crystalline, brown solid(103)(46mg) was formed, which was isolated by filtration.(Found: C,57.31; H,3.86. $C_{22}H_{18}O_8NCl$(monoacetate) requires C,57.45;H,3.94%), λ_{max}=262and392mμ (ε 28,200 and 4,920), ν_{CHCl_3}=1758(acetate), 1699(C=O),1644(amide) and 1567cm^{-1}(amide).

Zinc/Acetic Acid Reduction of (103)

The course of reaction was followed by ultraviolet spectroscopic analysis. The starting material (103)

had λ_{max} (acetic acid) = 262 and 394 mμ (ε 25,800 and 4,880). (103) (3.5 mg.) was dissolved in acetic acid (5 ml.) containing sodium acetate trihydrate (10 mg.). Zinc dust (50 mg.) was added and the mixture stirred under nitrogen at 30°. An aliquot was removed after 30 sec. which showed λ_{max} = 442 mμ, with a weak inflexion at λ = 390 mμ. Further stirring (6 hr.) gave λ_{max} = 445 mμ, (ε 4,970).

Methylation of (103)

(103) (35.4 mg.) was dissolved in ether (10 ml.) and a dry ethereal solution of diazomethane was added in large excess. The solution was evaporated at room temperature after 0.5 hr. to give a brown solid (34.6 mg.), which was chromatographed over buffered silica. Elution with (99:1) chloroform-acetone gave a light brown solid (21.3 mg.), which was dried by addition of benzene and subsequent distillation *in vacuo*. This compound did not "crystallise" from hot benzene. For analysis a warm benzene solution was filtered and carefully evaporated. (Found: C,58.78; H,5.39. $C_{23}H_{20}O_8NCl$ (monomethyl monoacetate) requires C,58.28; H,4.25%), λ_{max} = 268 and 367 mμ (ε 22,000 and 3,300), ν_{CHCl_3} = 1759 (acetate), 1694 (C=O), 1655cm^{-1} (amide).

5a,6-Anhydro-7-chlorotetracycline (Anhydroaureomycin)(96)

The method of Stephen *et al*[115] was used without modification, and repeatedly gave material of high quality in good yields. The preparation was most conveniently carried out on a 5g. scale.

7-Chlorotetracycline hydrochloride (5g.) was added to a saturated solution of hydrogen chloride in AnalaR methanol (100ml.) at 5°, and left to stand in a refrigerator at 0°. During 4 days the supernatant changed in colour from pale yellow to dark orange, and a large portion of the yellow starting-material had gone into solution. The material which remained undissolved was isolated by rapid filtration, washed with a little methanol, and allowed to air-dry to afford recovered 7-chlorotetracycline hydrochloride (2.08g.). The dark orange filtrate was reduced in volume to 20 ml. at 30° *in vacuo*, and then treated with dry diethyl ether to precipitate anhydroaureomycin hydrochloride (2.27g.) Conversion to the free base was effected by dissolution of the salt in water (250 ml.) followed by dropwise addition of sodium hydroxide (2N) until the pH of the solution was 4-5. The resulting amorphous precipitate was coagulated by warming the solution to 50°, isolated by filtration, washed with

water, and dried at 50° *in vacuo* (1.82g.). Complete removal of water was effected by the addition of benzene followed by azeotropic distillation. On cooling the benzene solution deposited orange needles of anhydroaureomycin (1.29g.). For analysis a sample was recrystallised from benzene. (Found: C,57.19; H,4.8; N,5.99. $C_{22}H_{21}N_2O_7Cl$ requires C,57.33; H,4.59; N,6.08%), λ_{max} = 273 and 435 mμ (ε 51,000 and 8,860), ν_{CHCl_3} = 3482s, 3367, 3300, 1649, 1622, 1586, 1568cm^{-1}.

Treatment of (96) with Lead Tetraacetate (PbTA)

(a) 4 Equiv. Lead Tetraacetate

(96) (115 mg., 0.25 mM) in acetic acid (5 ml.) was stirred at room temperature during the dropwise addition (0.25 hr.) of a solution of PbTA (443 mg., 1mM) in acetic acid (15 ml.). The first drop caused an intense green colouration, which thereafter intensified rendering the solution opaque. After 0.5 hr., iodometric titration (difficult owing to colour) indicated uptake of ca. 3.3 equiv. PbTA. The reaction mixture was poured into water and extracted with chloroform. Evaporation of the water-washed chloroform extracts yielded a brown solid (34.7 mg.), λ_{max} = 365-370 mμ (ε 5,500) and ν_{CHCl_3} = ca. 1740cm^{-1}. All attempts to crystallise this product failed.

(b) 2.5 Equiv. Lead Tetraacetate

(96) (460 mg., 1mM) in acetic acid (150 ml.) was stirred at room temperature during the dropwise addition of PbTA (1.1g., 2.5 Equiv.) in acetic acid (20 ml.) over 0.75 hr. Stirring was continued for a further hour, after which time a drop of the reaction mixture no longer darkened a moist potassium iodide test-paper. A fine suspension of dark solid was removed from the reaction mixture by filtration through celite. The dark red-brown filtrate was evaporated at 30° in vacuo. Final traces of acetic acid were removed by separate additions of benzene followed by azeotropic distillation. The product was virtually insoluble in warm benzene; trituration with boiling chloroform afforded a near-black, semi-solid gum, λ_{max} = 335 mμ (broad). This product was not further investigated.

The brown residue from the chloroform triturations was treated with methanolic hydrochloric acid (2N, 10 ml.) in the presence of chloroform (20 ml.). The reddish chloroform layer was isolated, and the aqueous layer re-extracted with chloroform (2 x 20 ml.). The combined chloroform extracts were washed with water and evaporated to give a brown non-crystalline solid, (110), (55 mg.), λ_{max} = 265-270 and 388-394 mμ (ε 13,800

and 4,200), ν_{CHCl_3} = 1741 (acetate), 1702 (C = O), 1643 (amide), 1599 (aromatic C = C) and 1572cm^{-1} (amide).

All attempts to crystallise the product failed.

Oxidation of (96) with Caro's Acid [H_2SO_5]

Preparation of Caro's Acid.

The preparation described by Fieser[116] was used.

Caro's acid was prepared by addition of finely-powdered potassium perdisulphate (10g., 30mM) to stirred ice-cold concentrated sulphuric acid (7 ml.). After an hour the reaction mixture had assumed a white paste-like consistency. Ice (40g.) was added with stirring, and the solution was stored at 0°. After the ice had melted, the clear solution was diluted with water to 50 ml., and estimated with 0.1N sodium thiosulphate (thio). The solution contained 27.8 mM Caro's acid. A portion (2 ml.) of this solution was diluted to 20 ml. with water, and re-estimated with 0.01N thio (0.058 equiv./ml.). The diluted solution was used directly in the experiments described below.

(a) In methanolic magnesium sulphate solution.

This procedure was patterned after a method of Bamberger.[53]

(96) (103 mg., 0.22 mM) was dissolved by prolonged

shaking in methanolic sulphuric acid (0.2N, 30 ml.). Sufficient powdered magnesium carbonate was added to render the stirred solution neutral; thereafter more magnesium carbonate (1g.) was added, giving a final pH of 9. To this stirred solution was added Caro's acid (4 ml. 0.232 mM). After 2 min. all the Caro's acid was consumed (KI test-paper). After stirring for an hour the solution was filtered to give an orange-brown solid, which, after drying, was Soxhlet-extracted with chloroform. The chloroform-soluble portion was starting material (λ_{max} = 440 mμ).

The orange-red aqueous filtrate (pH 8.9) was acidified and extracted with chloroform. Removal of solvent gave a brown solid having λ_{max} = 440 mμ. Thus no oxidation-product was detected.

(b) <u>In 0.2N Sulphuric acid</u>

(96) (121 mg., 0.265 mM) was shaken with sulphuric acid (0.2N, 30 ml.) but after 4 hr. only a small amount had dissolved. To this stirred suspension was added Caro's acid (5 ml. 0.27 mM) and the mixture shaken vigorously. After 12 hr. a KI-test was positive but weak. After 18 hr. the test was negative. The suspension had darkened somewhat. The dark brown solid obtained by prolonged filtration at the pump

was washed with water and dried in vacuo at 70° (88 mg.). It was shown to be starting material, λ_{max} = 273 and 440 mμ (ε 41,500 and 8,400).

The filtrate was yellow-orange, and had λ_{max} = 272 and 370-380 (broad) mμ - corresponding to 2 mg. of hydroxylated product. This solution was not investigated further.

This experiment was repeated using 3 equiv. Caro's acid, and the filtrate again showed λ_{max} = 370 - 380 (broad) mμ.

(c) In Aqueous Acetic Acid.

(96) (111 mg., 0.242 mM) was dissolved in acetic acid (20 ml.) and a solution of Caro's acid (0.9 ml., 0.242 mM) in acetic acid (3 ml.) was added dropwise with stirring. Tests made periodically with KI-strips appeared negative - until moistened. Accordingly water (5 ml.) was added to the reaction mixture; KI-tests were then positive without moistening. The homogeneous solution was allowed to stand until the KI-test was negative (20 hr.). A sample showed λ_{max} = 446 mμ. A further equiv. of Caro's acid was added, which was consumed overnight. A sample showed λ_{max} = 275, 390, and an inflexion at 430 mμ. The reaction mixture was reduced in volume to 2 ml., and adjusted to pH 7 with ethanolic ammonia solution.

The resultant mixture was evaporated *in vacuo* to give a brown residue, trituration of which with methanol gave a brown solution having λ_{max} = 375 - 385 mμ. Trituration with chloroform also gave a solution having a similar λ_{max}.

Photooxygenation Procedures.

(a) 100 mg. Scale (2 x 20 W. Lamps)

Two fluorescent lamps (20 W.) were disposed vertically each side of a 20cm. Pyrex glass tube of 30 ml. capacity. The tube was equipped with a B24 ground glass joint, which housed a centre down-tube fitted with a 0.5 cm. glass sinter at its extremity. In this way, oxygen could be percolated through the solution for periods as long as a month without undue loss of solvent.

(b) 1g. Scale (4 x 20 W. Lamps)

Experiments on a 1g. scale were carried out in a 1 l. round-bottomed B24 flask equipped with a centre down-tube,,as described above. Four fluorescent lamps (20 W.) were disposed horizontally about the flask - one at each side and two below the flask. Since the lamps were 60 cm. in length, three 1 l. flasks could be illuminated simultaneously.

(c) 700 mg. Scale (450 W. Lamp)

Latterly, the acquisition of a Hanovia Photochemical Reactor (Cat. No.05190 - Hanovia Lamps Division of Engelhard Industries Limited, Slough, Bucks.) greatly facilitated the photo-oxygenation studies. The immersion lamp of 450 Watts, which constituted the main feature of the apparatus, proved an outstandingly dramatic improvement on the original light-source; photo-oxygenations which had previously required 8-10 days could now be accomplished in 6 hr. The lamp was equipped with a glass sleeve, through which water was passed as coolant. The reaction flask (10 l) supplied by Hanovia was too large for convenient use. It was replaced by a 2.5 l. cylindrical open-ended flask, which was fitted at its rim with a cork ring - on which the lamp and sleeve were seated. The displacement-volume of the lamp and sleeve was large; 800 ml. solvent was sufficient to fill the flask. Oxygen was introduced into the solution via a glass tube with a sinter at its extremity.

Photo-oxygenations of Anhydro-7-chlorotetracycline(96)

(I) Using water-saturated Chloroform as solvent

(100 mg. scale)

Anhydroaureomycin (100 mg.) was dissolved in

oxygenated (see above). The reaction was followed spectroscopically. During the course of 6 days the λ_{max} = 446 mμ of the starting material diminished, giving way to a new λ_{max} = 370-380 (broad) mμ. The sinter and the walls of the reaction vessel had become coated with a brown deposit - some of which was soluble in chloroform. The solution was evaporated in vacuo. Residual water was removed by addition of benzene followed by azeotropic distillation. The product was a yellow-brown solid (92.1 mg.), having λ_{max} = 260 and 370-377 mμ (ε 21,000 and 3,700). Part of the product (86 mg.) in chloroform (10 ml.) was chromatographed over buffered silica (10g.) with the following results.

	Elutriant			Weight eluted in mg.
1.	Chloroform	100 ml.	- small orange band straight through	ca. 1
2.	ditto	100 ml.	- dark yellow eluate	28
		600 ml.	- ditto	
		200 ml.	- pale yellow eluate	
		200 ml.	- nearly colourless	
3.	Chloroform-dioxan (9:1)	150 ml.	yellow	8
4.	ditto (1:1)	200 ml.	"	
5.	Dioxan	200 ml.	"	
6.	Methanol	100 ml.		ca. 2
				39

Fraction 2, a dark brown solid, was dissolved in hot benzene (5 ml.) and filtered. On cooling a brown non-crystalline solid deposited (5.8 mg.), λ_{max} = 265 and 365-370 mμ (ε 21,000 and 4,200), ν_{CHCl_3} = 1742, 1711, 1644, 1600 and 1569cm^{-1}. The filtrate was evaporated (21 mg.), λ_{max} = 430 - 440 mμ.

The photo-oxygenation was repeated; again the "benzene-recrystallised" product showed ν_{CHCl_3} = 1740 and 1710cm^{-1}, and λ_{max} = 365 - 370 mμ.

The nature of this product was undetermined.

(II) Using AnalaR Benzene as solvent.

(a) 100 mg. Scale

A solution of (96) (100 mg.) in AnalaR benzene (30 ml.) was photo-oxygenated for 7 days. A sample of the reaction mixture did not give a hydroperoxide test, and showed λ_{max} = 440 mμ. This indicated that oxygenation had not occurred. However, on concentration of the solution a small amount of insoluble residue was observed; filtration afforded a yellow solid (6.7 mg.), λ_{max} = ca. 250 and 372-377 mμ. This material gave a positive hydroperoxide test.

The filtrate was photo-oxygenated further (3 days) to give more yellow solid (6.7 mg.). This product crystallised from chloroform. The total product (13 mg.) was recrystallised from chloroform to give

golden yellow needles (98) (6.5 mg.). (Found (slow combustion): C,53.38; H,3.93. $C_{22}H_{21}N_2O_9Cl$ requires C,53.64; H,4.30%) This experiment was repeated on a 1g. scale (see below)

(b) 1g. Scale

A solution of (96) (1g.) in AnalaR benzene (1 l.) was photo-oxygenated (see above). After 3 days no visible difference was apparent. After 4 days a yellow deposit was apparent on the base of the "bubbler", and also on the sides of the flask where the illumination was most intense and solvent had evaporated. The ensuing isolation procedure was carried out on the combined reaction products of 2 x 1g. photo-oxygenation experiments.

The benzene solution (ca. 2 l.) was reduced in volume to 100 ml., which caused deposition of yellow solid (780 mg.). The yellow solid which adhered to the surfaces of each of the reaction flasks was dissolved in boiling chloroform, and this solution was used to recrystallise the major product (780mg.). From this solution there was obtained yellow needles (98) (407 mg.). Found (slow combustion): C,53.01; H,4.11; N,5.68. $C_{22}H_{21}N_2O_9Cl$ requires C,53.64; H,4.03; N,5.69%) ν_{CHCl_3} = 3608, 3467, 1707, 1640, 1600 and 1580cm^{-1}, $[\alpha]_D$ (0.5% in 0.1N HCl) - 20°;

λ_{max} = 249 and 375-380 mμ (ε 24,100 and 4,510), λ_{max} (Ni^{2+}) = 410-415 mμ (ε 11,000). This product gave a positive ferrous thiocyanate reaction.

The benzene mother liquors were recycled to yield a further crop of hydroperoxide (720 mg.) giving a total yield of crude hydroperoxide of 1.5 g. (70%).

(c) 700 mg. Scale - 450 W Lamp.

(96) (700 mg.) was dissolved in benzene (700 ml.) and the solution photo-oxygenated for 6 hr. Golden-yellow crystals had formed abundantly on the bubbler-tube and on the Pyrex sleeve. The reaction-mixture was concentrated to 50 ml., and filtered (670 mg.) The product gave a positive hydroperoxide test, and contained no starting material (no λ_{max} > 400 mμ; only λ_{max} = 370-385 mμ). This represented a 90% conversion to 6-deoxy-6-hydroperoxy-5a,11a-dehydro-aureomycin (98). This material was not recrystallised owing to lack of time.

Hydrogenations of 7-chloro-6-deoxy-6-hydroperoxy-5, 5a -dehydrotetracycline (98)

(a) 3 Mol. H_2/Pd-C (10%)/1 Mol. Et_3N/MeOH

Palladium-charcoal (10%; 20 mg.) was pre-reduced in methanol (10 ml.) containing freshly distilled

triethylamine (3.67 mg., 36.6 μM). (98) (18.0 mg.) was added, and the mixture shaken in the presence of hydrogen. Uptake of 1 Mol. H_2 was complete after 2 min. The reduction was allowed to proceed until no more hydrogen was absorbed (80 min., 2.9 Mol. H_2). The filtered reaction mixture was poured into water and continuously extracted with benzene (24 hr.). The benzene extract was concentrated in vacuo to give a pale yellow solid (3 mg.), whose ultra-violet spectrum (λ_{max} = 268-269 and 360-363 mμ) was similar to that of tetracycline (λ_{max} = 268 and 361-363 mμ) (ε 18,500 and 15,300), a sample of which was prepared from tetracycline hydrochloride, and recrystallised from benzene.

(b) 1 Mol. H_2/Pd-C (10%)/MeOH

Preparation of 7-chloro-5, 5a -dehydrotetracycline(97)

Pd-C (10%; 64 mg.) was pre-reduced in methanol (10 ml.). (98) (60.7 mg.) was added and the mixture shaken in the presence of hydrogen. Uptake of 1 Mol. H_2 was complete in 150 sec., whereupon the reaction was stopped. The filtered reaction mixture was concentrated in vacuo to give a yellow solid (63 mg.), which did not give a positive hydroperoxide test. Dissolution in boiling sodium-dry diethyl ether (150 ml.), followed by concentration of the solution to 20 ml. afforded yellow

needles (10 mg.), having spectral properties consistent with its formulation as (97). λ_{max} = 254-256 and 385-390 mu (ε 23,800 and 5,220). λ_{max} (Ni^{++}) = 411-416 mu (ε 12,000), ν_{CHCl_3} = 3580, 3475, 3300, 1709 1641, 1602, 1570cm^{-1}; ν_{KBr} = 1709, 1642, 1593cm^{-1}. $[\alpha]_D$ (0.4% in $CHCl_3$) + 210°; (0.5% in 0.03N HCl) + 15°.

An authentic sample of the natural Streptomyces aureofaciens metabolite (97) was kindly provided by Drs. J.R.D. McCormick and S. Kushner (Lederle Laboratories, Pearl River, New York) in the form of its hydrochloride. Conversion to the required free base involved dissolution of the hydrochloride (56 mg.) in water (5 ml.) followed by the addition of sodium hydroxide (0.2N) until the pH of the solution was 7. The cloudy solution was extracted with chloroform (6 x 5 ml.), the combined extracts washed with water, and the dried (Na_2SO_4) solution concentrated *in vacuo*. The yellow solid (43 mg.) was crystallised by dissolution in diethyl ether followed by concentration *in vacuo*. λ_{max} = 250-258 and 385-390 mu (ε 21,800 and 5,060) $[\alpha]_D$ (0.38% in $CHCl_3$) + 212°. McCormick *et al*[38] report $[\alpha]_D$ (0.65% in 0.03N HCl) + 15.5°. The infrared spectrum (KBr disc) was superposable on that of the synthetic material.

Zinc - Acetic Acid Reduction of 7-chloro-5,5a-dehydrotetracycline (97)

Aromatisation of ring B to give 5a,6-anhydro-7-chlorotetracycline (96)

(97) (1 mg.) was dissolved in acetic acid (5 ml.), and the ultraviolet spectrum of the solution recorded: λ_{max} = 390-395 mμ. Sodium acetate trihydrate (1 crystal) and zinc dust (5 mg.) were added with shaking. The mixture was immediately filtered; the filtrate had λ_{max} = 415-420 mμ - the expected divalent ion bethochromic shift, c.f.[108]. Fresh zinc dust (5 mg.) was added with shaking. After 0.5 hr. the solution showed λ_{max} = 437-442 mμ, which indicated formation of 5a,6-anhydro-7-chlorotetracycline (96).

An authentic sample of (96) (λ_{max} = 440 mμ) when treated with Zn-Acetic acid for a similar period of time was recovered unchanged - although prolonged (2 hr.) contact with zinc resulted in the expected removal of the 12a-hydroxy grouping - as indicated by the advent of a maximum at λ = 390 mμ.

5a,6-Anhydrotetracycline, (96, H for Cl)

A generous sample of the above compound was kindly supplied by Dr. R.J. Boscott, Pfizer Limited, Sandwich, Kent. (Found (slow combustion): C,62.03; H,5.34; N,6.73. $C_{22}H_{22}N_2O_7$ requires C,61.69; H,5.20; N,6.57%. λ_{max} = 270 and 427-428 mμ (ε 55,000 and 9,800).)

Photo-oxygenation of Anhydrotetracycline (96, H for Cl) 700 mg. Scale / 450 W Lamp.

(96, H for Cl) (700 mg.) was dissolved in benzene (700 ml.) and photo-oxygenated (8 hr.). A yellow precipitate slowly deposited on the sides of the glass sleeve. This material gave a positive hydroperoxide test. The cloudy solution was filtered to give a yellow-brown solid (202 mg.) which also gave a hydroperoxide test. Ultraviolet spectroscopic analysis of this product indicated the presence of starting material (λ_{max} = 425 mμ) as well as the hydroperoxide λ_{max} = 362-372 mμ. An attempted fractionation of the product with benzene and chloroform was unsuccessful. The solid which coated the glass sleeve was not crystalline and was also a mixture of product and starting material. Lack of time prevented further investigation of this mixture.

5a,6-Anhydro-7-chloro-6-demethyltetracycline (100)

The details of this preparation were kindly supplied by Dr. R.K. Blackwood, Pfizer Limited.

7-Chloro-6-demethyltetracycline (2 g.) was heated under reflux with a mixture of methanol (30 ml.), concentrated hydrochloric acid (33 ml.) and water (17 ml.). After 0.5 hr. the pale yellow starting material had completely dissolved to give a pale orange solution;

further refluxing (1 hr.) caused the orange colouration to intensify. On gradual cooling a pale orange precipitate was formed, which was isolated by filtration. (Rapid cooling gave an amorphous product, which was difficult to filter quickly.) The pale yellow filtrate was refluxed (1 hr.) and allowed to cool. The precipitate formed was washed with a little methanol and combined with the first crop. The total crude product was dissolved in methanol (30 ml.) and a solution of triethylamine (6.5 ml.) in water (20 ml.) was added dropwise with manual agitation until the pH of the solution was 6. The cloudy solution was extracted with chloroform (4 x 50 ml.). The combined chloroform extracts were washed with water, and concentrated in vacuo. Benzene was added and evaporated to remove final traces of water. The product was an orange powder (965 mg.), which recrystallised from benzene as pale orange needles (100) (596 mg.). λ_{max} = 274, 332 and 430 mμ. A second crop (115 mg.) was obtained by concentration of the mother liquor.

Attempted Photo-oxygenation of (100)

390 mg. Scale / 450 W. Lamp.

(100) (390 mg.) was dissolved in benzene (800 ml.) and photo-oxygenated. After 20 hr. a brown solid had formed on the surface of the glass filter. Also the

solution was cloudy. Filtration - which, in view of the amorphous nature of the solid material, was slow - afforded a brown non-crystalline solid (68 mg.). This product had an ultraviolet spectrum similar to that of the starting-material (λ_{max} = 430 mμ), and showed no infrared absorption between 1650-1800cm^{-1}. Concentration *in vacuo* of a portion of the filtrate gave a pale orange residue which was indistinguishable from starting-material (λ_{max} = 430 mμ). Neither fraction gave a hydroperoxide test.

5a,6-Anhydro-5-hydroxytetracycline (Anhydroterramycin)(101)

This compound was previously prepared by Hochstein *et al*[117] by careful dehydration under acidic conditions of 5-hydroxytetracycline hydrochloride. Crystalline samples of the compound were reported to be obtainable only from aqueous acetone - with which it readily formed the acetone solvate $C_{22}H_{22}N_2O_8.CH_3COCH_3$. The following modification of the reported method gave crystalline material which analysed for an unsolvated product.

A solution of anhydrous hydrogen chloride (5.07 g.) in anhydrous acetone (200 ml.) cooled to 5° was poured over 5-hydroxytetracycline hydrochloride (5 g.; recrystallised from methanol). The temperature rose to 5° after 15 minutes and the solution was maintained at ca. 5° for 33 hr., after which time all of the

hydrochloride had dissolved to give a dark orange solution. Anhydrous ether was added to precipitate 3.9 g. of yellow product. Recrystallisation from n-butanol-dioxan (1:2; 70 ml.) - both solvents distilled from calcium hydride - yielded 5a,6-anhydro-5-hydroxytetracycline hydrochloride (1.8 g.). This product was dissolved in water (10 ml.) and the pH of the solution was rapidly adjusted to 5 with 5% sodium bicarbonate, whereupon the free base precipitated as an amorphous solid. Continuous extraction with benzene (24 hr.) gave an orange residue (1.2 g.). Dissolution of this product in AR benzene (30 ml.) followed by distillation at atmospheric pressure served to remove all the acetone as azeotrope. On gradual cooling, crystals of anhydro-5-hydroxytetracycline (101) deposited (960 mg.). (Found (slow combustion): C,59.96; H,5.14; N,6.40. $C_{22}H_{22}N_2O_8$ requires C,59.72; H,5.01; N,6.33%), λ_{max} (MeOH) = 269 and 425-426 mμ (ε 43,200 and 9,200).

Crystalline samples containing acetone of crystallisation could be freed of acetone by azeotropic distillation with benzene; the solvent-free product was crystallisable *via* seeding. Also, samples of (101) contaminated with the benzene-insoluble α- and β-apoterramycins could be purified by recrystallisation from benzene.

Attempted Photo-Oxygenation of Anhydroterramycin (101)

100 mg. Scale

Anhydroterramycin (101) (100 mg.) was dissolved in benzene (30 ml.) and photo-oxygenated (3 weeks). Some brown solid which was present in the reaction-mixture, was isolated by filtration (16 mg.). This material was intractable and displayed neither a characteristic ultraviolet spectrum nor an infrared spectrum in which any maxima were uniquely discernible. The compound did not give a hydroperoxide test.

m-Methoxyacetophenone (118)

The method of Johnson[127] was used.

Methylation of m-hydroxyacetophenone (50g.) yielded m-methoxyacetophenone (118) (35.1 g.), b.p. 135-136°/32 mm., $n_D^{21.5}$. = 1.5400, ν_{CCl_4} = 1682 (C = O) and 1036cm^{-1} (OCH_3), λ_{max} (EtOH) = 250 and 305 mμ (ε 8,020 and 2,700). [Lit.[127] b.p. 125-126°/12mm.]

o-Nitro-m-methoxyacetophenone (119)

The method of Simpson[128] was used.

o-nitro-m-methoxyacetophenone recrystallised from ethanol as pale yellow plates (37%), m.p.127-129°. [Lit.[128] 128-129°]

o-Amino-m-methoxyacetophenone (120)

The reduction conditions of Novack and Protiva[129] were used.

To a vigorously stirred solution of (119) (9.75 g., 50 mM) in aqueous ethanol (50%, 50 ml.) was added iron filings (10 g.), and dropwise a mixture of concentrated hydrochloric acid (1.4 ml.), ethanol (3 ml.) and water (3 ml.). The addition did not cause the mixture to reflux. The mixture was heated to reflux (2 hr.) and filtered hot through a fluted filter paper. The iron residues were washed with hot ethanol, and the combined filtrates were neutralised (1N sodium hydroxide) at 0° to give the amine as a green-yellow precipitate (7.65 g., 93%). Recrystallisation from ethanol-water gave lime green platelets, m.p. 63-64° [Lit.[128] 64-66°]

o-Cyano-m-methoxyacetophenone (121)

Prior to discovery of the reported[119] method (82% yield), we achieved a 23% yield utilising a general method described in Organic Syntheses.[130]

Sandmeyer Reaction

(120) (4.12 g., 25 mM) was dissolved at 40° in hydrochloric acid (24%, 5 ml.) and cooled to 0°. Sodium nitrite (1.9 g., 27.5 mM) was added slowly at 0° while the solution was manually swirled. The diazonium salt thus formed was neutralised (litmus) by the addition of solid sodium carbonate, and added dropwise to a stirred solution of cuprous cyanide (2.28 g., 26 mM) and sodium cyanide (3.5 g., 70 mM)

in water (10 ml.) held at 0°. The mixture was allowed to come to room temperature with stirring, and when nitrogen evolution ceased (1 hr.) the mixture was filtered. The crude product (2.9 g.) was only partially soluble in hot benzene. The insoluble portion (1.1 g.) was a purplish-coloured compound which was completely insoluble in all organic solvents. The infrared spectrum showed ν_{nuj} = 2130, 1690 and 1060cm^{-1}. Exploratory reactions on this inorganic complex utilised part of the product (250 mg.). The remainder (850 mg.) was dissolved in concentrated hydrochloric acid - giving a wine-red solution - diluted with water, and extracted with chloroform to give crude nitrile (466 mg.).

The benzene soluble portion was washed with 1N sodium hydroxide (2 x 20 ml.), and water, and evaporated to give reddish crystals of the nitrile (683 mg.).

The total crude nitrile (1.05 g.) was decolourised with charcoal and recrystallised from ethanol to give white crystals (825 mg.), m.p. 122-124°. A second recrystallisation gave white rectangular platelets (121), m.p. 124-124.5°. (Found: C,68.61; H,5.59; N,8.06. $C_{10}H_9O_2N$ requires C,68.56; H,5.18; N,8.00%), λ_{max} (EtOH) = 320-321 mμ (ε 9,670); ν_{CHCl_3} = 1696 (C = O), 2227 (CN) and 1060cm^{-1} (methyl ether). [Lit.[119] m.p. 123-124°]

Acidification of the sodium hydroxide extract and extraction with chloroform gave the phenolic by-product, o-hydroxy-m-methoxyacetophenone, as off-white crystals (323 mg.) m.p. 165-168° ν_{nuj} = 3300 (OH) and 1680 (C=O); λ_{max} = 217, 267, 277 and 345 mμ.

1,3-Diphenylpropan-1-ol (138)

Benzalacetophenone (137) (4.15 g.) was hydrogenated (2 mole H_2) in ethanol in the presence of 10% Pd-C (420 mg.). The crude oil (138) (4.19 g.) had ν_{film} = 3400cm^{-1} (OH), and was used unpurified in the next step.

trans-1,3-Diphenylprop-1-ene (139)

The crude alcohol (138) (4.19 g.) was heated (0.5 hr.) at 180°/25 mm. in the presence of p-toluene sulphonic acid (10 mg.). The olefin (139) distilled with very little fore-run as a colourless oil (2.48 g., 65%), b.p. 140-142°/1 mm., n_D^{25} = 1.5944. λ_{max} (EtOH) = 253 mμ; ν_{film} = 975cm^{-1} (trans C = C).

1,3-Diphenyl-1,2-oxide (140)

The method of preparation of perbenzoic acid and the epoxidation technique were taken from the review of Swern.[131]

To a solution of perbenzoic acid (1.4 g.) in chloroform (75 ml.) held at 0°, was added the olefin (139) (1.78 g.) in chloroform (20 ml.).

The mixture was washed with 2N sodium hydroxide and water, and dried over sodium sulphate. Evaporation gave a near-colourless oil, distillation of which gave the epoxide (140) as a colourless oil (914 mg., 48%), b.p. 138-140°/0.5 mm., n_D^{25} = 1.5681, ν_{film} = 870cm^{-1} (epoxide). [Levy[120] reported b.p. 162-165°/6 mm. and n_D^{18} = 1.575.]

A later fraction (352 mg., b.p. 150-152°/0.5 mm) had ν_{film} = 1710cm^{-1}, and its infrared spectrum was superposable on that of a pure sample of dibenzyl ketone (141).

Dibenzyl Ketone (141)

A commercial sample of dibenzyl ketone was recrystallised from diethyl ether - light petroleum to give white needles, m.p. 33-35° ν_{nujol} = 1712cm^{-1} (C = O).

1-Carbethoxy-1-cyano-2,2-dibenzylethylene (142)

This compound was prepared from dibenzyl ketone (52.5 g.) and ethyl cyanoacetate (34 g.) as described by Dufraisse.[122] The pale yellow viscous oil (142) (68 g.) b.p. 180-182°/0.02 mm., had ν_{film} = 2230(CN) and 1720cm^{-1} (ester).

Trituration of the distillation residues with diethyl ether yielded 3-benzyl-2-cyano-1-naphthol (147) as white platelets (1.2 g.), m.p. 210-213°.

Attempted Preparation of 1-carbethoxy-1-cyano-2,2-dibenzylethane (143)

The catalytic hydrogenation of (142) was attempted using the following catalyst/solvent combinations:-

a) Pd-C (10%)/EtOH-HCl

b) Pd-C (10%)/EtOH/3 atmosph. H_2

c) PtO_2/acetic acid

d) PtO_2/ethyl acetate.

In each case no absorption of hydrogen was observed and the starting material was recovered unchanged.

1,1-Dicyano-2,2-dibenzylethylene (142; CN for CO_2Et)

A crude sample of the above compound was prepared from dibenzyl ketone (141) and malononitrile by Dr. A.C. Rodriguez following the method of Dufraisse.[123] Crystallisation, and recrystallisation from pentane gave pure dinitrile as white platelets, m.p. 51-52° [Lit.[123] 50-51°] ν_{CCl_4} = 2233cm^{-1}; λ_{max} (MeOH) = 239 mμ (ε 12,030), λ_{max} (base) = 239 and 348 mμ. (ε 12,100 and 15,000).

1,1-Dicyano-2,2-dibenzylethane (143; CN for CO_2Et)

The unsaturated nitrile (142; CN for CO_2Et) (1 g.) was reduced (0.9 mol. H_2) in the presence of freshly reduced platinum oxide (250 mg.) in ethyl acetate (15 ml.). The product (1.02 g.) crystallised from ethanol to give white platelets (700 mg.), m.p. 110-111°, (Found:

C,83.07; H,6.11; N,10.52. $C_{18}H_{16}N_2$ requires C,83.04; H,6.20; N,10.76%), ν_{CCl_4} = 2256cm^{-1}, λ_{max} (MeOH) = 212 mμ (aromatic).

The action of Concentrated Sulphuric Acid on 1,1-Dicyano-2,2-dibenzylethane (143; CN for CO_2Et)

1,1-Dicyano-2,2-dibenzylethane (74.4 mg.) was dissolved in concentrated sulphuric acid (2 ml.) at 25° to give a colourless solution, which, after 2 hr., was poured onto excess water to give a yellow precipitate (41 mg.). A portion (35 mg.) of this product was dissolved in chloroform, and treated with pentane to give a yellow non-crystalline solid for analysis (Found: C,65.33; H,5.51; N,7.26%), ν_{nuj} = 3350, 3200, 2200, 1690, 1030 and 1010cm^{-1}. λ_{max}(EtOH) = 284 and 410 mμ (ε 22.1/mg. and 27.67/mg.); λ_{max}(ethanolic base) = 242 and 384 mμ (ε 38.6/mg. and 23.5/mg.).

The ultraviolet spectra were interconvertible in acid and base.

The nature of this product was not determined.

3-Benzyl-2-Cyano-1-Naphthol (144)

This compound was prepared *via* thermal cyclisation of 1-carbethoxy-1-cyano-2,2-dibenzylethylene (142), and was best carried out using acetamide as solvent as described by Dufraisse.[122] An alternative procedure using glycerol as solvent[132] was less successful.

(a) Heating with Glycerol at 240-250°

(142) (5 g.) was added to glycerol (20 ml.) and kept at 240-250° for 3 hours. After cooling, the reaction mixture was poured into ice-water (200 ml.) and allowed to age overnight at 0°. Filtration afforded a gummy solid, recrystallisation of which from ethanol yielded white platelets (144) (1.5 g.), m.p. 210-215°.

(b) Heating with Acetamide at 220°

(142) (5 g.) was added to acetamide (15 g.) and kept at 220° for 2 hours. The crude product, isolated as in (a), was a near-white solid (144), (2.8 g.), m.p. 211-214°.

Experiment (b) was repeated on a larger scale using (142) (58 g.) and acetamide (174 g.) to yield (144) (22.5 g.). For analysis a sample was sublimed at 180°/0.01 mm., m.p. 214-215° [Lit.[122] 212-213°], (Found: C,83.52; H,5.34. $C_{18}H_{13}NO$ requires C,83.37; H,5.05%), λ_{max} = 249, 290, 334 and 348 mμ (ε 46,600, 4,300, 4,480 and 5,680), λ_{max} (base) = 266 and 355 mμ (ε 71,600 and 7,770), ν_{nujol} = 3350 (OH) and 2230cm^{-1} (CN).

Chroman-3-one (128)

Samples of chroman-3-one (128) were recrystallised at 0° from light petroleum to give pale yellow rhombs, m.p. 38-39° [Lit.[125] 39°]

7-Hydroxyindanone (129)

This compound was prepared in 73% yield from chroman-3-one (128) (10 g.) and aluminium chloride (18 g.) according to the method of London;[125] recovery in steam afforded pale yellow needles (129), m.p. 108-110°; λ_{max} = 275 and 318 mμ (ε 12,500 and 3,300); ν_{nujol} = 3400 (OH) and 1680cm^{-1} (C = O). [Lit.[125] m.p. 111°]

7-Methoxyindanone (130)

This compound was prepared according to the method of London.[125] It was found essential to stir the reaction mixture in order to effect complete methylation. Recrystallisation of 7-methoxyindanone (130) from benzene-light petroleum gave pale yellow platelets, m.p. 102-103°; λ_{max} = 276 and 312 mμ. ν_{nujol} = 1690cm^{-1} (C = O). [Lit.[125] m.p. 102-103°]

3,5-Dimethoxybenzaldehyde (122)

This compound was prepared in 48% overall yield in a four-stage process (Stevens reaction) from 3,5-dimethoxybenzoic acid according to the method of Adams.[126] The crude product was conveniently purified via the preparation of the bisulphite addition compound. For analysis a sample was recrystallised from light petroleum to yield chunky rhombs, m.p. 44-46°. (Found: C,65.21; H,5.67. $C_9H_{10}O_3$ requires C,65.05; H,6.07%), λ_{max} (EtOH) = 269-270

and 325 mμ (ε 6,780 and 2,580) ν_{CHCl_3} = 2728 and 1703cm^{-1}.

2-(3,5-dimethoxybenzylidene)-7-methoxyindanone (131)

A solution of sodium hydroxide (0.4 g.) in ethanol (5 ml.) was added to a solution of 7-methoxyindanone (130) (1.89 g.) and 3,5-dimethoxybenzaldehyde (122) (1.94g.) in ethanol (50 ml.) and the resulting solution allowed to stand at room temperature for 2 days. The crude crystalline product (2.45 g.) which had precipitated was isolated by filtration. (The filtrate did not yield a further crop of product after 3 days - nor after reducing the volume of the solution to 10 ml.). Recrystallisation from ethanol gave pale yellow platelets (2.00 g.), m.p. 167-169° (Found: C,73.10; H,5.90. $C_{19}H_{18}O_4$ requires C,73.53; H,5.85%), λ_{max} = 325 (inflexion) and 345 mμ (ε 20,500 and 22,400), ν_{nujol} = 1682 (C = O), 1640 (C = C), 1065 (O CH_3) and 1050cm^{-1} (OCH_3).

2-(3,5-dimethoxybenzylidene)-7-hydroxyindanone

(131; H for Me)

A solution of sodium hydroxide (4.0 g.) in water (5 ml.) was added to a solution of 7-hydroxyindanone (129) (2.96 g.) and 3,5-dimethoxybenzaldehyde (122) (3.32 g.) in ethanol (50 ml.). The resulting

homogeneous yellow-orange solution was allowed to stand at room temperature for 3 hours. During this time a yellow solid gradually deposited, subsequent analysis showing it to be a mixture of the desired condensation product and its corresponding sodium salt. This crude mixture (2.1 g.) was slurried in aqueous ethanol and neutralised by the dropwise addition of acetic acid. The crude phenol (1.34 g.) was isolated therefrom by extraction with chloroform, and recrystallised from benzene to yield pale yellow platelets, (131; H for Me) (1.07 g.), m.p. 179-180°, (Found: C,73.19; H,5.43. $C_{18}H_{16}O_4$ requires C,72.96; H,5.44%), λ_{max} (EtOH) = 324 and 347 mμ; (ethanolic base) = 310 and 418 mμ, ν_{nujol} = 3300 (OH), 1670 (C = O), 1640 (C = C), 1075 (OCH_3) and 1055cm^{-1} (OCH_3).

2-(3,5-Dimethoxybenzyl)-7-methoxyindanone (132)

(131) (260 mg.) was hydrogenated in acetic acid (10 ml.) in the presence of Adam's catalyst (32 mg.). After the absorption of 1 mol. H_2 (20 min.) the reaction mixture was filtered and concentrated in vacuo to yield the crude product (226 mg.). A sample was recrystallised from benzene-light petroleum to yield platelets (132), λ_{max} = 259 and 312 mμ, ν_{nujol} = 1690cm^{-1} (C = O).

References.

References

The following abbreviations have been employed:

JACS. for Journal of the American Chemical Society.

J. for Journal of the Chemical Society.

Also those references which refer to publications by four or more authors have been conveniently abbreviated by quoting the first-named author only.

1. Broschard et al, Science, 1949, 109, 199.
2. Finlay et al, ibid 1950, 111, 85.
3. Stephens et al, JACS., 1952, 74, 4976.
4. Hochstein et al, ibid 1953, 75, 5435.
5. Conover et al, ibid 1953, 75, 4622.
6. Minieri et al, in "Antibiotics Annual", ed. by Welch, Medical Encyclopaedia Inc., New York, 1953-1954, p. 81.
7. Doerschuk et al, JACS., 1956, 78, 1508.
8. McCormick et al, ibid 1957, 79, 4561.
9. Stephens et al, ibid 1958, 80, 532 .
10. McCormick et al, ibid 1957, 79, 2849.
11. McCormick et al, ibid 1960, 82 3381.
12. Conover, in "Symposium on Antibiotics and Mould Metabolites," Chem. Soc. Publications No. 5, London, 1956, p. 48-81.

13. Albert, Nature, 1953, 172, 201.

14. Stephens et al JACS., 1956, 78, 4155.

15. Barrett, J. Pharm. Sci., 1963, 52, 310.

16. Donohue et al, JACS., 1963, 85, 851.

17. Robertson et al, ibid 1952, 74, 841.

18. Waller et al, ibid 1952, 74, 4981.

19. Green and Boothe, ibid 1960, 82, 3950.

20. Kende et al, ibid 1961, 83, 440.

21. Shemyakin et al, Tet. Lett., 1962, 20, 901.

22. Shemyakin et al, in "Chemistry of Antibiotics", U.S.S.R. Academy of Sciences, Vol. 1, Moscow, 1961, p. 180-268.

23. van Tamelen, Fortschr. Org. Naturstoffe, 1958, 16, 90.

24. Regna, in "Antibiotics: Their Chemistry and Non-medical Uses", ed. Goldberg, van Nostrand Co., Princeton, N.J., 1959, p. 77-96.

25. Vogel, Sci. Pharm, 1956, 24, 257.

26. To be published by Pergamon Institute, Headington Hall, Oxford - mentioned in ref. 13.

27. Boothe, Kushner and Williams, JACS., 1953, 75, 3623.

28. Boothe et al, ibid, 1959, 81, 1006.

29. Muxfeldt, Chem. Ber., 1959, 92, 3122.

30. Fields, Kende and Boothe, JACS., 1960, 82, 1250; Ibid 1961, 83, 4612.

31. Muxfeldt and Krentzer, Chem. Ber., 1961, 94, 881.

32. Holmlund, Andres and Shay, JACS., 1959, 81, 4748.

33. Conover et al, ibid 1962, 84, 3222.

34. Doerschuk et al, ibid 1956, 78, 1508.

35. Doerschuk et al, ibid 1959, 81, 3609.

36. Perelman et al, ibid 1961, 83, 4481.

37. McCormick et al, ibid 1957, 79, 4561.

38. McCormick et al, ibid 1958, 80, 5572.

39. McCormick et al, ibid 1958, 80, 6460.

40. Hochstein et al, ibid 1960, 82, 5934.

41. Miller and Hochstein, J. Org. Chem, 1962, 27, 2525.

42. Robinson, The Structural Relations of Natural Products, Clarendon Press, Oxford, 1955, p. 58.

43. Woodward, Angew. Chem. 1956, 68, 13.

44. Birch, Snell and Thomson, J., 1962, 425.

45. Snell, Unpublished experiments - quoted in ref.44.

46. Wakil, JACS., 1958, 80, 6465.

47. Bentley and Keil, Proc. Chem. Soc., 1961, 111.

48. cf. Birch, Proc. Chem. Soc., 1962, 3.

49. Gatenbeck, Biochem. Biophys. Res. Commun., 1961, 6, 422.

50. Muxfeldt, Buhr, and Bangert, Angew. Chem., (Internat. Ed.), 1962, 1, 157.

51. Bamberger, Ber., 1900, 33, 3600.

52. Bamberger, Ann., 1912, 390, 163.

53. Bamberger, Ber., 1903, 36, 2028.

54. London, Progress in Organic Chemistry (ed. Cook), 1961, 5, 46.

55. Wessely, Mh. Chem., 1953, 84, 124.

56. Wessely et al, ibid 1957, 88, 1067.

57. Hecker, Chem, Ber., 1959, 92, 1386.

58. Gold and Schwenk, JACS., 1958, 80, 5683.

59. Cosgrove and Waters, J., 1951, 388.

60. Woodward and Doering, JACS., 1945, 67, 860.

61. Hecker and Lattrell, Ann., 1963, 662, 48.

62. Hecker and Mueller, J. Biol. Chem., 1958, 233, 991.

63. Isler et al, Helv. Chim. Acta., 1963, 46, 636.

64. Goodwin and Witkop, JACS., 1957, 79, 179.

65. Metlesics, Wessely and Budzikiewicz, Tetrahedron, 1959, 6, 345.

66. Davies, Hassall and Schofield, Chem. and Ind., 1963, 740.

67. Cavill et al, J., 1954, 2785.

68. Hassall and Scott, in "Recent Developments in the Chemistry of Natural Phenolic Compounds", ed. by Ollis, Pergamon, 1961, p. 119.

69. Barton and Cohen, Festschrift Arthur Stoll, Birkhauser, Basel, 1957, p. 117.

70. Davies, Organic Peroxides, Butterworths, London, 1961.

71. Davies, Foster and White, J., 1954, 2204.

72. Dufraisse, Etienne and Rigaudy, Bull. Soc. chim. Fr., 1948, 804.

73. Bickel and Gersmann, Proc. Chem. Soc., 1957, 231.

74. Coppinger, JACS., 1957, 79, 2758.

75. Coppinger, ibid 1953, 75, 734.

76. Kharasch and Joshi, J. Org. Chem., 1957, 22, 1439.

77. Kharasch et al, ibid 1952, 17, 207.

78. Ref. 42. p 10

79. Scott et al, (in press).

80. Leete and Ghosal, Tet. Lett., 1962, 1179.

81. Wessely et al, Mh. Chem., 1954, 85, 69.

82. Dr. A.S. Kende, Lederle Laboratories, American Cyanimid Co., New York - private communication.

83. Julian et al, JACS., 1934, 56, 2174; 1935, 57, 1607, 2508; 1945, 67, 1721, 1724.

84. c.f. Dufraisse, XIV I.C.P.A.C., Experientia Supple., 1955, II, 27.

85. Dufraisse and Priou, Bull. Soc. chim. Fr., 1939, 6, 1649.

86. Badger, Structure and Reactions of Aromatic Compounds, Cambridge University Press, 1954, p.364.

87. Backstrom and Beatty, J. Phys. Chem., 1931, 35, 2530.

88. Rickards, in ref. 68. p. 4.

89. Thomson, Naturally Occurring Quinones, Butterworths, London, 1957.

90. Dr. T. Money, Nature, 1963, 199, 592 - whom I warmly thank for much helpful discussion.

91. Birch, Fortschr. Chem. Org. Naturstoffe, 1957, 14, 186.

92. Thomas, Biochem. J., 1961, 78, 748.

93. Gatenbeck, Svensk. Kem. Tidskr., 1960, 72, 188.

94. Stickings and Mahmoodian, Chem. and Ind., 1962, 1718.

95. Reference 70, p. 143 et seq.

96. Feigl, Spot Tests in Organic Analysis, 6th Edn., Elsevier, London, 1960, p. 535.

97. Friedel and Orchin, Ultraviolet spectra of Aromatic Compounds, John Wiley and Sons Inc., New York.

98. Bellamy, The Infrared Spectra of Complex Molecules, Methuen, London, 1954.

99. Schonberg and Mustafa, J., 1945, 657.

100. Ref. 12. p. 52.

101. Holmlund, Andres and Shay, JACS., 1959, 81, 4750.

102. McOmie, Advances in Organic Chemistry, ed. Raphael, Taylor and Wynberg, Interscience, Vol. 1, p. 191.

103. Craigee et al, Newer Methods of Preparative Organic Chemistry, Interscience, 1948, p.1.

104. Muxfeldt, Chem. Ber., 1961, 94, 881.

105. idem, ibid, 1961, 94, 892.

106. Schenck, Angew. Chem., 1952, 64, 12.

107. Schenck and Ziegler, Naturwiss, 1944, 32, 157; Schenck, Kinkel and Meitens, Ann., 1953, 584, 125.

108. Ref. 12, p. 62.

109. McCormick et al, JACS., 1958, 80, 5572.

110. Scott and Bedford, JACS,,1962, 84, 2271.

111. McCormick et al, ibid, 1962, 84, 3024.

112. M. Scach von Wittenau, Chas. Pfizer and Co., Groton, U.S.A. - private communication.

113. Drs. J.H. Boothe, A.S. Kende and T.L. Fields, Lederle Laboratories, American Cyanimid Co., New York - private communication.

114. Naylor and Gardner, JACS., 1953, 53, 4114.

115. Stephen et al, ibid, 1954, 76, 3574.

116. Fieser, Experiments in Organic Chemistry, D.C. Heath and Co., Boston, 1955, p. 308.

117. Hochstein et al, JACS., 1953, 75, 5470.

118. Bhati, Tetrahedron, 1962, 18, 1519.

119. Kushner et al, JACS., 1953, 75, 1097.

120. Levy, Bull. Soc. chim. Fr., 1931, [4], 49, 1765.

121. Parker and Isaacs, Chem. Rev., 1959, 59, 737.

122. Dufraisse and Etienne, Bull. Soc. chim. Fr., 1949, 779; 1955, 265.

123. Dufraisse, Comp. Rend., 1954, 239, 1744.

124. Cope, JACS., 1941, 63, 3452.

125. London and Razdan , J., 1954, 4301.

126. Adams, JACS., 1948, 70, 664.

127. Johnson, ibid, 1952, 74, 2843.

128. Simpson, J., 1945, 643.

129. Novack and Protiva, Coll. Czech., 1959, 24, 3966.

130. Organic Syntheses, Chapman and Hall, 1941, Coll. Vol. I, p. 514.

131. Swern, Organic Reactions, 1953, 7, 378.

132. Marion, Can. J. Res., 1940, 18B, 265; 1937, 15B, 480.

133. Muxfeldt, Chem. Ber., 1956, 89, 1393.